



## INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

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<b>(21) International Application Number:</b> PCT/EP00/01978  <b>(22) International Filing Date:</b> 07 March 2000 (07.03.2000)  <b>(30) Priority Data:</b> 09/265,149      09 March 1999 (09.03.1999)    US  <b>(60) Parent Application or Grant</b> NOVARTIS AG [/]; (). NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H. [/]; (). SALMERON, John, Manuel [/]; (). WEISLO, Laura, Jean [/]; (). WILLITS, Michael, G. [/]; (). MENGISTE, Tesfaye [/]; (). SALMERON, John, Manuel [/]; (). WEISLO, Laura, Jean [/]; (). WILLITS, Michael, G. [/]; (). MENGISTE, Tesfaye [/]; (). BECKER, Konrad ; ().		<b>Published</b>
<b>(54) Title: NOVEL PLANT GENES AND USES THEREOF</b> <b>(54) Titre: NOUVEAUX GENES DE VEGETAUX ET LEURS UTILISATIONS</b>  <b>(57) Abstract</b>  Homologues of the Arabidopsis NIM1 gene, which is involved in the signal transduction cascade leading to systemic acquired resistance (SAR), are isolated from Nicotiana tabacum (tobacco), Lycopersicon esculentum (tomato), Brassica napus (oilseed rape), Arabidopsis thaliana, Beta vulgaris (sugarbeet), Helianthus annuus (sunflower), and Solanum tuberosum (potato). The invention further concerns transformation vectors and processes for expressing the NIM1 homologues in transgenic plants to increase SAR gene expression and enhance broad spectrum disease resistance.  <b>(57) Abrégé</b>  L'invention concerne des homologues du gène Arabidopsis NIM1, impliqué dans la cascade de transduction des signaux menant à la résistance systémique acquise (RSA), qui sont isolés à partir de Nicotiana tabacum (tabac), de Lycopersicon esculentum (tomate), de Brassica napus (colza oléagineux), d'Arabidopsis thaliana, de Beta vulgaris (betterave à sucre), d'Helianthus annuus (tournesol) et de Solanum tuberosum (pomme de terre). L'invention concerne également des vecteurs de transformation et des processus permettant d'exprimer les homologues de NIM1 dans des végétaux transgéniques afin d'accroître l'expression du gène RSA et d'élargir le large spectre de résistance aux maladies.		

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<b>(21) International Application Number:</b> PCT/EP00/01978 <b>(22) International Filing Date:</b> 7 March 2000 (07.03.00)  <b>(30) Priority Data:</b> 09/265,149                      9 March 1999 (09.03.99)                      US  <b>(71) Applicant (for all designated States except AT US):</b> NOVARTIS AG [CH/CH]; Schwarzwaldallee 215, CH-4058 Basel (CH).  <b>(71) Applicant (for AT only):</b> NOVARTIS-ERFINDUNGEN VERWALTUNGSGESELLSCHAFT M.B.H. [AT/AT]; Brunner Strasse 59, A-1230 Vienna (AT).  <b>(72) Inventors; and</b> <b>(75) Inventors/Applicants (for US only):</b> SALMERON, John, Manuel [US/US]; 1308 Blackberry Lane, Hillsborough, NC 27278 (US). WEISLO, Laura, Jean [US/US]; 914 West South Street, Raleigh, NC 27603 (US). WILLITS, Michael, G. [US/US]; 804 Winter Hill Drive, Apex, NC 27502 (US). MENGISTE, Tesfaye [ET/US]; 4516-G Emerald Forest Drive, Durham, NC 27713 (US).		<b>(74) Agent:</b> BECKER, Konrad; Novartis AG, Corporate Intellectual Property, Patent & Trademark Department, CH-4002 Basel (CH).  <b>(81) Designated States:</b> AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CR, CU, CZ, DE, DK, DM, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MA, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, TZ, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).  <b>Published</b> <i>Without international search report and to be republished upon receipt of that report.</i>
<b>(54) Title:</b> NOVEL PLANT GENES AND USES THEREOF		
<b>(57) Abstract</b>  Homologues of the <i>Arabidopsis NIM1</i> gene, which is involved in the signal transduction cascade leading to systemic acquired resistance (SAR), are isolated from <i>Nicotiana tabacum</i> (tobacco), <i>Lycopersicon esculentum</i> (tomato), <i>Brassica napus</i> (oilseed rape), <i>Arabidopsis thaliana</i> , <i>Beta vulgaris</i> (sugarbeet), <i>Helianthus annuus</i> (sunflower), and <i>Solanum tuberosum</i> (potato). The invention further concerns transformation vectors and processes for expressing the <i>NIM1</i> homologues in transgenic plants to increase SAR gene expression and enhance broad spectrum disease resistance.		

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**Description**

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**NOVEL PLANT GENES AND USES THEREOF**

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The present invention relates to broad-spectrum disease resistance in plants, including the phenomenon of systemic acquired resistance (SAR). More particularly, the present invention relates to the identification, isolation and characterization of homologues of the *Arabidopsis NIM1* gene involved in the signal transduction cascade leading to systemic acquired resistance in plants.

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Plants are constantly challenged by a wide variety of pathogenic organisms including viruses, bacteria, fungi, and nematodes. Crop plants are particularly vulnerable because they are usually grown as genetically-uniform monocultures; when disease strikes, losses can be severe. However, most plants have their own innate mechanisms of defense against pathogenic organisms. Natural variation for resistance to plant pathogens has been identified by plant breeders and pathologists and bred into many crop plants. These natural disease resistance genes often provide high levels of resistance to or immunity against pathogens.

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Systemic acquired resistance (SAR) is one component of the complex system plants use to defend themselves from pathogens (Hunt and Ryals, 1996; Ryals *et al.*, 1996). See also, U.S. Patent No. 5,614,395. SAR is a particularly important aspect of plant-pathogen responses because it is a pathogen-inducible, systemic resistance against a broad spectrum of infectious agents, including viruses, bacteria, and fungi. When the SAR signal transduction pathway is blocked, plants become more susceptible to pathogens that normally cause disease, and they also become susceptible to some infectious agents that would not normally cause disease (Gaffney *et al.*, 1993; Delaney *et al.*, 1994; Delaney *et al.*, 1995; Delaney, 1997; Bi *et al.*, 1995; Mauch-Mani and Slusarenko, 1996). These observations indicate that the SAR signal transduction pathway is critical for maintaining plant health.

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Conceptually, the SAR response can be divided into two phases. In the initiation phase, a pathogen infection is recognized, and a signal is released that travels through the phloem to distant tissues. This systemic signal is perceived by target cells, which react by expression of both SAR genes and disease resistance. The maintenance phase of SAR refers to the period of time, from weeks up to the entire life of the plant, during which the plant is in a quasi steady state, and disease resistance is maintained (Ryals *et al.*, 1996).

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5 Salicylic acid (SA) accumulation appears to be required for SAR signal transduction. Plants that cannot accumulate SA due to treatment with specific inhibitors, epigenetic repression of phenylalanine ammonia-lyase, or transgenic expression of salicylate hydroxylase, which specifically degrades SA, also cannot induce either SAR gene  
10 expression or disease resistance (Gaffney *et al.*, 1993; Delaney *et al.*, 1994; Mauch-Mani and Slusarenko, 1996; Maher *et al.*, 1994; Pallas *et al.*, 1996). Although it has been suggested that SA might serve as the systemic signal, this is currently controversial and, to date, all that is known for certain is that if SA cannot accumulate, then SAR signal  
15 transduction is blocked (Pallas *et al.*, 1996; Shulaev *et al.*, 1995; Vernooij *et al.*, 1994).

Recently, *Arabidopsis* has emerged as a model system to study SAR (Uknes *et al.*, 1992; Uknes *et al.*, 1993; Cameron *et al.*, 1994; Mauch-Mani and Slusarenko, 1994; Dempsey and Klessig, 1995). It has been demonstrated that SAR can be activated in  
20 *Arabidopsis* by both pathogens and chemicals, such as SA, 2,6-dichloroisonicotinic acid (INA) and benzo(1,2,3)thiadiazole-7-carbothioic acid S-methyl ester (BTH) (Uknes *et al.*, 1992; Vernooij *et al.*, 1995; Lawton *et al.*, 1996). Following treatment with either INA or  
25 BTH or pathogen infection, at least three pathogenesis-related (PR) protein genes, namely, PR-1, PR-2, and PR-5 are coordinately induced concomitant with the onset of resistance (Uknes *et al.*, 1992, 1993). In tobacco, the best characterized species, treatment with a pathogen or an immunization compound induces the expression of at least nine sets of  
30 genes (Ward *et al.*, 1991). Transgenic disease-resistant plants have been created by transforming plants with various SAR genes (U.S. Patent No. 5,614,395).

A number of *Arabidopsis* mutants have been isolated that have modified SAR signal transduction (Delaney, 1997). The first of these mutants are the so-called *lsd* (lesions  
35 simulating disease) mutants and *acd2* (accelerated cell death) (Dietrich *et al.*, 1994; Greenberg *et al.*, 1994). These mutants all have some degree of spontaneous necrotic lesion formation on their leaves, elevated levels of SA, mRNA accumulation for the SAR genes, and significantly enhanced disease resistance. At least seven different *lsd* mutants  
40 have been isolated and characterized (Dietrich *et al.*, 1994; Weymann *et al.*, 1995). Another interesting class of mutants are *cim* (constitutive immunity) mutants (Lawton *et al.*, 1993). *See also*, U.S. Patent No. 5,792,904 and International PCT Application WO  
45 94/16077. Like *lsd* mutants and *acd2*, *cim* mutants have elevated SA and SAR gene expression and resistance, but in contrast to *lsd* or *acd2*, do not display detectable lesions on their leaves. *cpr1* (constitutive expresser of PR genes) may be a type of *cim* mutant;

5 however, because the presence of microscopic lesions on the leaves of *cpr1* has not been ruled out, *cpr1* might be a type of *lsd* mutant (Bowling *et al.*, 1994).

10 Mutants have also been isolated that are blocked in SAR signaling. *ndr1* (non-race-specific disease resistance) is a mutant that allows growth of both *Pseudomonas syringae* containing various avirulence genes and also normally avirulent isolates of *Peronospora parasitica* (Century *et al.*, 1995). Apparently this mutant is blocked early in SAR signaling. *npr1* (nonexpresser of PR genes) is a mutant that cannot induce expression of the SAR signaling pathway following INA treatment (Cao *et al.*, 1994). *eds* (enhanced disease susceptibility) mutants have been isolated based on their ability to support bacterial infection following inoculation of a low bacterial concentration (Glazebrook *et al.*, 1996; Parker *et al.*, 1996). Certain *eds* mutants are phenotypically very similar to *npr1*, and, recently, *eds5* and *eds53* have been shown to be allelic to *npr1* (Glazebrook *et al.*, 1996). *nim1* (noninducible immunity) is a mutant that supports *P. parasitica* (i.e., causal agent of downy mildew disease) growth following INA treatment (Delaney *et al.*, 1995; U.S. Patent No. 5,792,904). Although *nim1* can accumulate SA following pathogen infection, it cannot induce SAR gene expression or disease resistance, suggesting that the mutation blocks the pathway downstream of SA. *nim1* is also impaired in its ability to respond to INA or BTH, suggesting that the block exists downstream of the action of these chemicals (Delaney *et al.*, 1995; Lawton *et al.*, 1996).

30 Allelic *Arabidopsis* genes have been isolated and characterized, mutants of which are responsible for the *nim1* and *npr1* phenotypes, respectively (Ryals *et al.*, 1997; Cao *et al.*, 1997). The wild-type *NIM1* gene product is involved in the signal transduction cascade leading to both SAR and gene-for-gene disease resistance in *Arabidopsis* (Ryals *et al.*, 1997). Ryals *et al.*, 1997 also report the isolation of five additional alleles of *nim1* that show a range of phenotypes from weakly impaired in chemically induced PR-1 gene expression and fungal resistance to very strongly blocked. Transformation of the wild-type *NPR1* gene into *npr1* mutants not only complemented the mutations, restoring the responsiveness of SAR induction with respect to PR-gene expression and disease resistance, but also rendered the transgenic plants more resistant to infection by *P. syringae* in the absence of SAR induction (Cao *et al.*, 1997). WO 98/06748 describes the isolation of *NPR1* from *Arabidopsis* and a homologue from *Nicotiana glutinosa*. See also, WO 97/49822, WO 98/26082, and WO 98/29537.

5           Despite much research and the use of sophisticated and intensive crop protection measures, including genetic transformation of plants, losses due to disease remain in the billions of dollars annually. Therefore, there is a continuing need to develop new crop protection measures based on the ever-increasing understanding of the genetic basis for  
10           disease resistance in plants. In particular, there is a need for the identification, isolation, and characterization of homologues of the *Arabidopsis NIM1* gene from additional species of plants.

15           In describing the present invention, the following terms will be employed, and are intended to be defined as indicated below.

          Associated With / Operatively Linked: Refers to two DNA sequences that are related physically or functionally. For example, a promoter or regulatory DNA sequence is said to  
20           be "associated with" a DNA sequence that codes for an RNA or a protein if the two sequences are operatively linked, or situated such that the regulator DNA sequence will affect the expression level of the coding or structural DNA sequence.

25           Chimeric Gene: A recombinant DNA sequence in which a promoter or regulatory DNA sequence is operatively linked to, or associated with, a DNA sequence that codes for an mRNA or which is expressed as a protein, such that the regulator DNA sequence is able to regulate transcription or expression of the associated DNA sequence. The regulator  
30           DNA sequence of the chimeric gene is not normally operatively linked to the associated DNA sequence as found in nature.

          Coding Sequence: a nucleic acid sequence that is transcribed into RNA such as mRNA, rRNA, tRNA, snRNA, sense RNA or antisense RNA. Preferably the RNA is then  
35           translated in an organism to produce a protein.

          Complementary: refers to two nucleotide sequences that comprise antiparallel nucleotide sequences capable of pairing with one another upon formation of hydrogen  
40           bonds between the complementary base residues in the antiparallel nucleotide sequences.

          Expression: refers to the transcription and/or translation of an endogenous gene or a transgene in plants. In the case of antisense constructs, for example, expression may refer to the transcription of the antisense DNA only.

45           Expression Cassette: A nucleic acid sequence capable of directing expression of a particular nucleotide sequence in an appropriate host cell, comprising a promoter operatively linked to the nucleotide sequence of interest which is operatively linked to termination signals. It also typically comprises sequences required for proper translation of  
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5 the nucleotide sequence. The expression cassette comprising the nucleotide sequence of  
interest may be chimeric, meaning that at least one of its components is heterologous with  
respect to at least one of its other components. The expression cassette may also be one  
10 which is naturally occurring but has been obtained in a recombinant form useful for  
heterologous expression. Typically, however, the expression cassette is heterologous with  
respect to the host, i.e., the particular nucleic acid sequence of the expression cassette  
does not occur naturally in the host cell and must have been introduced into the host cell or  
an ancestor of the host cell by a transformation event. The expression of the nucleotide  
15 sequence in the expression cassette may be under the control of a constitutive promoter or  
of an inducible promoter which initiates transcription only when the host cell is exposed to  
some particular external stimulus. In the case of a multicellular organism, such as a plant,  
the promoter can also be specific to a particular tissue, or organ, or stage of development.

20 Gene: A defined region that is located within a genome and that, besides the  
aforementioned coding nucleic acid sequence, comprises other, primarily regulatory, nucleic  
acid sequences responsible for the control of the expression, that is to say the transcription  
and translation, of the coding portion. A gene may also comprise other 5' and 3'  
25 untranslated sequences and termination sequences. Further elements that may be present  
are, for example, introns.

Heterologous DNA Sequence: The terms "heterologous DNA sequence",  
30 "exogenous DNA segment" or "heterologous nucleic acid," as used herein, each refer to a  
sequence that originates from a source foreign to the particular host cell or, if from the same  
source, is modified from its original form. Thus, a heterologous gene in a host cell includes  
a gene that is endogenous to the particular host cell but has been modified through, for  
35 example, the use of DNA shuffling. The terms also includes non-naturally occurring multiple  
copies of a naturally occurring DNA sequence. Thus, the terms refer to a DNA segment  
that is foreign or heterologous to the cell, or homologous to the cell but in a position within  
the host cell nucleic acid in which the element is not ordinarily found. Exogenous DNA  
40 segments are expressed to yield exogenous polypeptides.

Homologous DNA Sequence: A DNA sequence naturally associated with a host cell  
into which it is introduced.

45 Isocoding: A nucleic acid sequence is isocoding with a reference nucleic acid  
sequence when the nucleic acid sequence encodes a polypeptide having the same amino  
acid sequence as the polypeptide encoded by the reference nucleic acid sequence.

5 Isolated: In the context of the present invention, an isolated nucleic acid molecule or an isolated enzyme is a nucleic acid molecule or enzyme that, by the hand of man, exists apart from its native environment and is therefore not a product of nature. An isolated nucleic acid molecule or enzyme may exist in a purified form or may exist in a non-native  
10 environment such as, for example, a recombinant host cell.

Minimal Promoter: promoter elements, particularly a TATA element, that are inactive or that have greatly reduced promoter activity in the absence of upstream activation. In the presence of a suitable transcription factor, the minimal promoter functions to permit  
15 transcription.

Native: refers to a gene that is present in the genome of an untransformed cell.

Naturally occurring: the term "naturally occurring" is used to describe an object that can be found in nature as distinct from being artificially produced by man. For example, a protein or nucleotide sequence present in an organism (including a virus), which can be isolated from a source in nature and which has not been intentionally modified by man in the laboratory, is naturally occurring.  
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25 *NIM1*: Gene described in Ryals *et al.*, 1997, which is involved in the SAR signal transduction cascade.

NIM1: Protein encoded by the *NIM1* gene

30 Nucleic acid: the term "nucleic acid" refers to deoxyribonucleotides or ribonucleotides and polymers thereof in either single- or double-stranded form. Unless specifically limited, the term encompasses nucleic acids containing known analogues of natural nucleotides which have similar binding properties as the reference nucleic acid and are metabolized in a manner similar to naturally occurring nucleotides. Unless otherwise indicated, a particular  
35 nucleic acid sequence also implicitly encompasses conservatively modified variants thereof (*e.g.* degenerate codon substitutions) and complementary sequences and as well as the sequence explicitly indicated. Specifically, degenerate codon substitutions may be achieved by generating sequences in which the third position of one or more selected (or all) codons is substituted with mixed-base and/or deoxyinosine residues (Batzer *et al.*,  
40 *Nucleic Acid Res.* 19: 5081 (1991); Ohtsuka *et al.*, *J. Biol. Chem.* 260: 2605-2608 (1985); Rossolini *et al.*, *Mol. Cell. Probes* 8: 91-98 (1994)). The terms "nucleic acid" or "nucleic acid sequence" may also be used interchangeably with gene, cDNA, and mRNA encoded by a gene. In the context of the present invention, the nucleic acid molecule is preferably a segment of DNA. Nucleotides are indicated by their bases by the following standard abbreviations: adenine (A), cytosine (C), thymine (T), and guanine (G).  
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5 ORF: Open Reading Frame.

Plant: Any whole plant.

10 Plant Cell: Structural and physiological unit of a plant, comprising a protoplast and a cell wall. The plant cell may be in form of an isolated single cell or a cultured cell, or as a part of higher organized unit such as, for example, a plant tissue, a plant organ, or a whole plant.

15 Plant Cell Culture: Cultures of plant units such as, for example, protoplasts, cell culture cells, cells in plant tissues, pollen, pollen tubes, ovules, embryo sacs, zygotes and embryos at various stages of development.

20 Plant Material: Refers to leaves, stems, roots, flowers or flower parts, fruits, pollen, egg cells, zygotes, seeds, cuttings, cell or tissue cultures, or any other part or product of a plant.

Plant Organ: A distinct and visibly structured and differentiated part of a plant such as a root, stem, leaf, flower bud, or embryo.

25 Plant tissue: A group of plant cells organized into a structural and functional unit. Any tissue of a plant *in planta* or in culture is included. This term includes, but is not limited to, whole plants, plant organs, plant seeds, tissue culture and any groups of plant cells organized into structural and/or functional units. The use of this term in conjunction with, or in the absence of, any specific type of plant tissue as listed above or otherwise embraced by this definition is not intended to be exclusive of any other type of plant tissue.

30 Promoter: An untranslated DNA sequence upstream of the coding region that contains the binding site for RNA polymerase II and initiates transcription of the DNA. The promoter region may also include other elements that act as regulators of gene expression.

35 Protoplast: An isolated plant cell without a cell wall or with only parts of the cell wall.

40 Purified: the term "purified," when applied to a nucleic acid or protein, denotes that the nucleic acid or protein is essentially free of other cellular components with which it is associated in the natural state. It is preferably in a homogeneous state although it can be in either a dry or aqueous solution. Purity and homogeneity are typically determined using analytical chemistry techniques such as polyacrylamide gel electrophoresis or high performance liquid chromatography. A protein which is the predominant species present in a preparation is substantially purified. The term "purified" denotes that a nucleic acid or protein gives rise to essentially one band in an electrophoretic gel. Particularly, it means that the nucleic acid or protein is at least about 50% pure, more preferably at least about 85% pure, and most preferably at least about 99% pure.

5                   Recombinant DNA molecule: a combination of DNA molecules that are joined together using recombinant DNA technology

10                   Regulatory Elements: Sequences involved in controlling the expression of a nucleotide sequence. Regulatory elements comprise a promoter operably linked to the nucleotide sequence of interest and termination signals. They also typically encompass sequences required for proper translation of the nucleotide sequence.

15                   Selectable marker gene: a gene whose expression in a plant cell gives the cell a selective advantage. The selective advantage possessed by the cells transformed with the selectable marker gene may be due to their ability to grow in the presence of a negative selective agent, such as an antibiotic or a herbicide, compared to the growth of non-transformed cells. The selective advantage possessed by the transformed cells, compared to non-transformed cells, may also be due to their enhanced or novel capacity to utilize an added compound as a nutrient, growth factor or energy source. Selectable marker gene also refers to a gene or a combination of genes whose expression in a plant cell gives the cell both, a negative and a positive selective advantage.

20                   Significant Increase: an increase in enzymatic activity that is larger than the margin of error inherent in the measurement technique, preferably an increase by about 2-fold or greater of the activity of the wild-type enzyme in the presence of the inhibitor, more preferably an increase by about 5-fold or greater, and most preferably an increase by about 10-fold or greater.

25                   The terms "identical" or percent "identity" in the context of two or more nucleic acid or protein sequences, refer to two or more sequences or subsequences that are the same or have a specified percentage of amino acid residues or nucleotides that are the same, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection.

30                   Substantially identical: the phrase "substantially identical," in the context of two nucleic acid or protein sequences, refers to two or more sequences or subsequences that have at least 60%, preferably 80%, more preferably 90-95%, and most preferably at least 99% nucleotide or amino acid residue identity, when compared and aligned for maximum correspondence, as measured using one of the following sequence comparison algorithms or by visual inspection. Preferably, the substantial identity exists over a region of the sequences that is at least about 50 residues in length, more preferably over a region of at least about 100 residues, and most preferably the sequences are substantially identical over at least about 150 residues. In a most preferred embodiment, the sequences are

5 substantially identical over the entire length of the coding regions. Furthermore,  
substantially identical nucleic acid or protein sequences perform substantially the same  
function.

10 For sequence comparison, typically one sequence acts as a reference sequence to  
which test sequences are compared. When using a sequence comparison algorithm, test  
and reference sequences are input into a computer, subsequence coordinates are  
designated if necessary, and sequence algorithm program parameters are designated. The  
15 sequence comparison algorithm then calculates the percent sequence identity for the test  
sequence(s) relative to the reference sequence, based on the designated program  
parameters.

20 Optimal alignment of sequences for comparison can be conducted, e.g., by the  
local homology algorithm of Smith & Waterman, *Adv. Appl. Math.* 2: 482 (1981), by the  
homology alignment algorithm of Needleman & Wunsch, *J. Mol. Biol.* 48: 443 (1970), by the  
search for similarity method of Pearson & Lipman, *Proc. Nat'l. Acad. Sci. USA* 85: 2444  
(1988), by computerized implementations of these algorithms (GAP, BESTFIT, FASTA, and  
25 TFASTA in the Wisconsin Genetics Software Package, Genetics Computer Group, 575  
Science Dr., Madison, WI), or by visual inspection (*see generally*, Ausubel *et al.*, *infra*).

30 One example of an algorithm that is suitable for determining percent sequence identity  
and sequence similarity is the BLAST algorithm, which is described in Altschul *et al.*, *J. Mol.*  
*Biol.* 215: 403-410 (1990). Software for performing BLAST analyses is publicly available  
through the National Center for Biotechnology Information (<http://www.ncbi.nlm.nih.gov/>).  
This algorithm involves first identifying high scoring sequence pairs (HSPs) by identifying  
35 short words of length W in the query sequence, which either match or satisfy some  
positive-valued threshold score T when aligned with a word of the same length in a  
database sequence. T is referred to as the neighborhood word score threshold (Altschul *et*  
*al.*, 1990). These initial neighborhood word hits act as seeds for initiating searches to find  
40 longer HSPs containing them. The word hits are then extended in both directions along  
each sequence for as far as the cumulative alignment score can be increased. Cumulative  
scores are calculated using, for nucleotide sequences, the parameters M (reward score for  
a pair of matching residues; always > 0) and N (penalty score for mismatching residues;  
45 always < 0). For amino acid sequences, a scoring matrix is used to calculate the cumulative  
score. Extension of the word hits in each direction are halted when the cumulative  
alignment score falls off by the quantity X from its maximum achieved value, the cumulative  
score goes to zero or below due to the accumulation of one or more negative-scoring  
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5 residue alignments, or the end of either sequence is reached. The BLAST algorithm  
parameters W, T, and X determine the sensitivity and speed of the alignment. The BLASTN  
program (for nucleotide sequences) uses as defaults a wordlength (W) of 11, an  
10 expectation (E) of 10, a cutoff of 100, M=5, N=-4, and a comparison of both strands. For  
amino acid sequences, the BLASTP program uses as defaults a wordlength (W) of 3, an  
expectation (E) of 10, and the BLOSUM62 scoring matrix (see Henikoff & Henikoff, *Proc.*  
*Natl. Acad. Sci. USA* 89: 10915 (1989)).

15 In addition to calculating percent sequence identity, the BLAST algorithm also  
performs a statistical analysis of the similarity between two sequences (see, e.g., Karlin &  
Altschul, *Proc. Nat'l. Acad. Sci. USA* 90: 5873-5787 (1993)). One measure of similarity  
provided by the BLAST algorithm is the smallest sum probability (P(N)), which provides an  
20 indication of the probability by which a match between two nucleotide or amino acid  
sequences would occur by chance. For example, a test nucleic acid sequence is considered  
similar to a reference sequence if the smallest sum probability in a comparison of the test  
nucleic acid sequence to the reference nucleic acid sequence is less than about 0.1, more  
25 preferably less than about 0.01, and most preferably less than about 0.001.

Another indication that two nucleic acid sequences are substantially identical is that  
the two molecules hybridize to each other under stringent conditions. The phrase  
"hybridizing specifically to" refers to the binding, duplexing, or hybridizing of a molecule only  
30 to a particular nucleotide sequence under stringent conditions when that sequence is  
present in a complex mixture (e.g., total cellular) DNA or RNA. "Bind(s) substantially" refers  
to complementary hybridization between a probe nucleic acid and a target nucleic acid and  
embraces minor mismatches that can be accommodated by reducing the stringency of the  
35 hybridization media to achieve the desired detection of the target nucleic acid sequence.

"Stringent hybridization conditions" and "stringent hybridization wash conditions" in the  
context of nucleic acid hybridization experiments such as Southern and Northern  
40 hybridizations are sequence dependent, and are different under different environmental  
parameters. Longer sequences hybridize specifically at higher temperatures. An extensive  
guide to the hybridization of nucleic acids is found in Tijssen (1993) *Laboratory Techniques*  
*in Biochemistry and Molecular Biology-Hybridization with Nucleic Acid Probes* part I chapter  
45 2 "Overview of principles of hybridization and the strategy of nucleic acid probe assays"  
Elsevier, New York. Generally, highly stringent hybridization and wash conditions are  
selected to be about 5°C lower than the thermal melting point ( $T_m$ ) for the specific sequence

5 at a defined ionic strength and pH. Typically, under "stringent conditions" a probe will hybridize to its target subsequence, but to no other sequences.

10 The  $T_m$  is the temperature (under defined ionic strength and pH) at which 50% of the target sequence hybridizes to a perfectly matched probe. Very stringent conditions are selected to be equal to the  $T_m$  for a particular probe. An example of stringent hybridization conditions for hybridization of complementary nucleic acids which have more than 100 complementary residues on a filter in a Southern or northern blot is 50% formamide with 1 mg of heparin at 42°C, with the hybridization being carried out overnight. An example of highly stringent wash conditions is 0.1 M NaCl at 72°C for about 15 minutes. An example of stringent wash conditions is a 0.2x SSC wash at 65°C for 15 minutes (*see*, Sambrook, *infra*, for a description of SSC buffer). Often, a high stringency wash is preceded by a low stringency wash to remove background probe signal. An example medium stringency wash for a duplex of, e.g., more than 100 nucleotides, is 1x SSC at 45°C for 15 minutes. An example low stringency wash for a duplex of, e.g., more than 100 nucleotides, is 4-6x SSC at 40°C for 15 minutes. For short probes (e.g., about 10 to 50 nucleotides), stringent conditions typically involve salt concentrations of less than about 1.0M Na ion, typically about 0.01 to 1.0 M Na ion concentration (or other salts) at pH 7.0 to 8.3, and the temperature is typically at least about 30°C. Stringent conditions can also be achieved with the addition of destabilizing agents such as formamide. In general, a signal to noise ratio of 2x (or higher) than that observed for an unrelated probe in the particular hybridization assay indicates detection of a specific hybridization. Nucleic acids that do not hybridize to each other under stringent conditions are still substantially identical if the proteins that they encode are substantially identical. This occurs, e.g., when a copy of a nucleic acid is created using the maximum codon degeneracy permitted by the genetic code.

35 The following are examples of sets of hybridization/wash conditions that may be used to clone homologous nucleotide sequences that are substantially identical to reference nucleotide sequences of the present invention: a reference nucleotide sequence preferably hybridizes to the reference nucleotide sequence in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 2X SSC, 0.1% SDS at 50°C, more desirably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 1X SSC, 0.1% SDS at 50°C, more desirably still in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 0.5X SSC, 0.1% SDS at 50°C, preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 0.1X

5           SSC, 0.1% SDS at 50°C, more preferably in 7% sodium dodecyl sulfate (SDS), 0.5 M NaPO<sub>4</sub>, 1 mM EDTA at 50°C with washing in 0.1X SSC, 0.1% SDS at 65°C.

10           A further indication that two nucleic acid sequences or proteins are substantially identical is that the protein encoded by the first nucleic acid is immunologically cross reactive with, or specifically binds to, the protein encoded by the second nucleic acid. Thus, a protein is typically substantially identical to a second protein, for example, where the two proteins differ only by conservative substitutions.

15           The phrase "specifically (or selectively) binds to an antibody," or "specifically (or selectively) immunoreactive with," when referring to a protein or peptide, refers to a binding reaction which is determinative of the presence of the protein in the presence of a heterogeneous population of proteins and other biologics. Thus, under designated immunoassay conditions, the specified antibodies bind to a particular protein and do not bind in a significant amount to other proteins present in the sample. Specific binding to an antibody under such conditions may require an antibody that is selected for its specificity for a particular protein. For example, antibodies raised to the protein with the amino acid sequence encoded by any of the nucleic acid sequences of the invention can be selected to obtain antibodies specifically immunoreactive with that protein and not with other proteins except for polymorphic variants. A variety of immunoassay formats may be used to select antibodies specifically immunoreactive with a particular protein. For example, solid-phase ELISA immunoassays, Western blots, or immunohistochemistry are routinely used to select monoclonal antibodies specifically immunoreactive with a protein. See Harlow and Lane (1988) *Antibodies, A Laboratory Manual*, Cold Spring Harbor Publications, New York ("Harlow and Lane"), for a description of immunoassay formats and conditions that can be used to determine specific immunoreactivity. Typically a specific or selective reaction will be at least twice background signal or noise and more typically more than 10 to 100 times background.

40           "Conservatively modified variations" of a particular nucleic acid sequence refers to those nucleic acid sequences that encode identical or essentially identical amino acid sequences, or where the nucleic acid sequence does not encode an amino acid sequence, to essentially identical sequences. Because of the degeneracy of the genetic code, a large number of functionally identical nucleic acids encode any given polypeptide. For instance the codons CGT, CGC, CGA, CGG, AGA, and AGG all encode the amino acid arginine. Thus, at every position where an arginine is specified by a codon, the codon can be altered to any of the corresponding codons described without altering the encoded protein. Such



5 nucleic acid variations are "silent variations" which are one species of "conservatively  
modified variations." Every nucleic acid sequence described herein which encodes a protein  
also describes every possible silent variation, except where otherwise noted. One of skill will  
10 recognize that each codon in a nucleic acid (except ATG, which is ordinarily the only codon  
for methionine) can be modified to yield a functionally identical molecule by standard  
techniques. Accordingly, each "silent variation" of a nucleic acid which encodes a protein is  
implicit in each described sequence.

15 Furthermore, one of skill will recognize that individual substitutions deletions or  
additions that alter, add or delete a single amino acid or a small percentage of amino acids  
(typically less than 5%, more typically less than 1%) in an encoded sequence are  
"conservatively modified variations," where the alterations result in the substitution of an  
20 amino acid with a chemically similar amino acid. Conservative substitution tables providing  
functionally similar amino acids are well known in the art. The following five groups each  
contain amino acids that are conservative substitutions for one another: Aliphatic: Glycine  
(G), Alanine (A), Valine (V), Leucine (L), Isoleucine (I); Aromatic: Phenylalanine (F),  
25 Tyrosine (Y), Tryptophan (W); Sulfur-containing: Methionine (M), Cysteine (C); Basic:  
Arginine (R), Lysine (K), Histidine (H); Acidic: Aspartic acid (D), Glutamic acid (E),  
Asparagine (N), Glutamine (Q). *See also*, Creighton (1984) *Proteins*, W.H. Freeman and  
Company. In addition, individual substitutions, deletions or additions which alter, add or  
30 delete a single amino acid or a small percentage of amino acids in an encoded sequence  
are also "conservatively modified variations."

A "subsequence" refers to a sequence of nucleic acids or amino acids that comprise a  
35 part of a longer sequence of nucleic acids or amino acids (e.g., protein) respectively.

Nucleic acids are "elongated" when additional nucleotides (or other analogous  
40 molecules) are incorporated into the nucleic acid. Most commonly, this is performed with a  
polymerase (e.g., a DNA polymerase), e.g., a polymerase which adds sequences at the 3'  
terminus of the nucleic acid.

Two nucleic acids are "recombined" when sequences from each of the two nucleic acids  
are combined in a progeny nucleic acid. Two sequences are "directly" recombined when both of  
45 the nucleic acids are substrates for recombination. Two sequences are "indirectly recombined"  
when the sequences are recombined using an intermediate such as a cross-over  
oligonucleotide. For indirect recombination, no more than one of the sequences is an actual  
substrate for recombination, and in some cases, neither sequence is a substrate for  
50 recombination.

5 A "specific binding affinity" between two molecules, for example, a ligand and a receptor, means a preferential binding of one molecule for another in a mixture of molecules. The binding of the molecules can be considered specific if the binding affinity is about  $1 \times 10^4 \text{ M}^{-1}$  to about  $1 \times 10^6 \text{ M}^{-1}$  or greater.

10 Transformation: a process for introducing heterologous DNA into a host cell or organism.

15 "Transformed," "transgenic," and "recombinant" refer to a host organism such as a bacterium or a plant into which a heterologous nucleic acid molecule has been introduced. The nucleic acid molecule can be stably integrated into the genome of the host or the nucleic acid molecule can also be present as an extrachromosomal molecule. Such an extrachromosomal molecule can be auto-replicating. Transformed cells, tissues, or plants are understood to encompass not only the end product of a transformation process, but also transgenic progeny thereof. A "non-transformed," "non-transgenic," or "non-recombinant" host refers to a wild-type organism, e.g., a bacterium or plant, which does not contain the heterologous nucleic acid molecule.

25 The present invention addresses the aforementioned needs by providing several homologues of the *Arabidopsis NIM1* gene from additional species of plants. In particular, the present invention concerns the isolation of *Nicotiana tabacum* (tobacco), *Lycopersicon esculentum* (tomato), *Brassica napus* (oilseed rape), *Arabidopsis thaliana*, *Beta vulgaris* (sugarbeet), *Helianthus annuus* (sunflower), and *Solanum tuberosum* (potato) homologues of the *NIM1* gene, which encode proteins believed to be involved in the signal transduction cascade responsive to biological and chemical inducers that lead to systemic acquired resistance in plants.

30 Hence, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that encodes SEQ ID NO:2, 4, 6, 8, 16, 18, 20, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 62, 64, 66, 68, 70, 72, or 74.

35 In another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73.

45 In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that comprises an at least 20, 25, 30, 35, 40, 45, or 50 (preferably 20) consecutive base pair portion identical in sequence to an at least 20, 25, 30, 35, 40, 45, or 50 (preferably 20) consecutive base pair portion of SEQ ID NO:1, 3, 5, 7, 15,

5 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73.

10 In still another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a *Lycopersicon esculentum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, SEQ ID NO:26 and 28, or SEQ ID NO:59 and 60.

15 In yet another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a *Beta vulgaris* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:22 and 24 or SEQ ID NO:26 and 28.

20 In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a *Helianthus annuus* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:26 and 28.

25 In another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a *Solanum tuberosum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:21 and 24, SEQ ID NO:21 and 23, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, or SEQ ID NO:26 and 28.

30 In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from a *Brassica napus* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10 or SEQ ID NO:26 and 28.

35 In yet another embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from an *Arabidopsis thaliana* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:13 and 14, SEQ ID NO:21 and 24, or SEQ ID NO:22 and 24.

40 In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from an *Nicotiana tabacum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10, SEQ ID NO:11 and 12, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, or SEQ ID NO:26 and 28; or

5 In a further embodiment, the present invention is directed to an isolated nucleic acid molecule comprising a nucleotide sequence that can be amplified from an plant DNA library using the polymerase chain reaction with a pair of primers comprising the first 20  
10 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73.

15 The present invention also encompasses a chimeric gene comprising a promoter active in plants operatively linked to a *NIM1* homologue coding sequence of the present invention, a recombinant vector comprising such a chimeric gene, wherein the vector is capable of being stably transformed into a host, as well as a host stably transformed with such a vector. Preferably, the host is a plant such as one of the following agronomically  
20 important crops: rice, wheat, barley, rye, canola, sugarcane, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape,  
25 raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum, and sugarcane. The present invention also encompasses seed from a plant of the invention.

30 Further, the present invention is directed to a method of increasing SAR gene expression in a plant by expressing in the plant a chimeric gene that itself comprises a promoter active in plants operatively linked to a *NIM1* homologue coding sequence of the present invention, wherein the encoded protein is expressed in the transformed plant at  
35 higher levels than in a wild type plant.

40 In addition, the present invention is directed to a method of enhancing disease resistance in a plant by expressing in the plant a chimeric gene that itself comprises a promoter active in plants operatively linked to a *NIM1* homologue coding sequence of the present invention, wherein the encoded protein is expressed in the transformed plant at  
45 higher levels than in a wild type plant.

Further, the present invention is directed to a PCR primer selected from the group consisting of SEQ ID NO:9-14, 21-28, 59, and 60.

50 The present invention also encompasses a method for isolating a *NIM1* homologue involved in the signal transduction cascade leading to systemic acquired resistance in plants comprising amplifying a DNA molecule from a plant DNA library using the polymerase chain reaction with a pair of primers corresponding to the first 20 nucleotides and the

5 reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID  
NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63,  
65, 67, 69, 71, or 73 or with the pair of primers set forth as SEQ ID NO:9 and 10, SEQ ID  
10 NO:11 and 12, SEQ ID NO:13 and 14, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ  
ID NO:21 and 23, SEQ ID NO:25 and 28, SEQ ID NO:26 and 28, or SEQ ID NO:59 and 60.  
In a preferred embodiment, the plant DNA library is a *Nicotiana tabacum* (tobacco),  
*Lycopersicon esculentum* (tomato), *Brassica napus* (oilseed rape), *Arabidopsis thaliana*,  
*Beta vulgaris* (sugarbeet), *Helianthus annuus* (sunflower), or *Solanum tuberosum* (potato)  
15 DNA library.

Northern data on several of the *NIM1* homologues described herein indicates  
constitutive expression or BTH-inducibility. The homologues of the *NIM1* gene described  
20 herein are predicted to encode proteins involved in the signal transduction cascade  
responsive to biological and chemical inducers, which leads to systemic acquired resistance  
in plants. The present invention also concerns the transgenic expression of such *NIM1*  
homologues in plants to increase SAR gene expression and enhance disease resistance.

25 The DNA sequences of the invention can be isolated using the techniques described  
in the examples below, or by PCR using the sequences set forth in the sequence listing as  
the basis for constructing PCR primers. For example, oligonucleotides having the  
sequence of approximately the first and last 20-25 consecutive nucleotides of SEQ ID NO:7  
30 (e.g., nucleotides 1-20 and 1742-1761 of SEQ ID NO:7) can be used as PCR primers to  
amplify the cDNA sequence (SEQ ID NO:7) directly from a cDNA library from the source  
plant (*Arabidopsis thaliana*). The other DNA sequences of the invention can likewise be  
amplified by PCR from cDNA or genomic DNA libraries of the respective plants using the  
35 ends of the DNA sequences set forth in the sequence listing as the basis for PCR primers.

The transgenic expression of the *NIM1* homologues of the invention in plants is  
predicted to result in immunity to a wide array of plant pathogens, which include, but are not  
40 limited to viruses or viroids, e.g. tobacco or cucumber mosaic virus, ringspot virus or  
necrosis virus, pelargonium leaf curl virus, red clover mottle virus, tomato bushy stunt virus,  
and like viruses; fungi, e.g. oomycetes such as *Phytophthora parasitica* and *Peronospora*  
*tabacina*; bacteria, e.g. *Pseudomonas syringae* and *Pseudomonas tabaci*; insects such as  
45 aphids, e.g. *Myzus persicae*; and lepidoptera, e.g., *Heliothis spp.*; and nematodes, e.g.,  
*Meloidogyne incognita*. The vectors and methods of the invention are useful against a  
number of disease organisms of maize including but not limited to downy mildews such as  
*Sclerophthora macrospora*, *Sclerophthora rayissiae*, *Sclerospora graminicola*,  
50

5 *Peronosclerospora sorghi*, *Peronosclerospora philippinensis*, *Peronosclerospora sacchari*  
and *Peronosclerospora maydis*; rusts such as *Puccinia sorghi*, *Puccinia polysora* and  
*Physopella zaeae*; other fungi such as *Cercospora zaeae-maydis*, *Colletotrichum graminicola*,  
10 *Fusarium monoliforme*, *Gibberella zaeae*, *Exserohilum turcicum*, *Kabatiellu zaeae*, *Erysiphe*  
*graminis*, *Septoria* and *Bipolaris maydis*; and bacteria such as *Erwinia stewartii*.

The methods of the present invention can be utilized to confer disease resistance to  
a wide variety of plants, including gymnosperms, monocots, and dicots. Although disease  
resistance can be conferred upon any plants falling within these broad classes, it is  
15 particularly useful in agronomically important crop plants, such as rice, wheat, barley, rye,  
rape, corn, potato, carrot, sweet potato, sugar beet, bean, pea, chicory, lettuce, cabbage,  
cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper,  
celery, carrot, squash, pumpkin, zucchini, cucumber, apple, pear, quince, melon, plum,  
20 cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple,  
avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

A *NIM1* homologue coding sequence of the present invention may be inserted into  
25 an expression cassette designed for plants to construct a chimeric gene according to the  
invention using standard genetic engineering techniques. The choice of specific regulatory  
sequences such as promoter, signal sequence, 5' and 3' untranslated sequences, and  
enhancer appropriate for the achieving the desired pattern and level of expression in the  
30 chosen plant host is within the level of skill of the routineer in the art. The resultant  
molecule, containing the individual elements linked in proper reading frame, may be  
inserted into a vector capable of being transformed into a host plant cell.

35 Examples of promoters capable of functioning in plants or plant cells (i.e., those  
capable of driving expression of associated coding sequences such as those coding for  
*NIM1* homologues in plant cells) include the *Arabidopsis* and maize ubiquitin promoters;  
cauliflower mosaic virus (CaMV) 19S or 35S promoters and CaMV double promoters; rice  
40 actin promoters; PR-1 promoters from tobacco, *Arabidopsis*, or maize; nopaline synthase  
promoters; small subunit of ribulose biphosphate carboxylase (ssuRUBISCO) promoters,  
and the like. Especially preferred is the *Arabidopsis* ubiquitin promoter. The promoters  
themselves may be modified to manipulate promoter strength to increase expression of the  
45 associated coding sequence in accordance with art-recognized procedures. Preferred  
promoters for use with the present invention are those that confer high level constitutive  
expression.

5           Signal or transit peptides may be fused to the *NIM1* homologue coding sequence in the chimeric DNA constructs of the invention to direct transport of the expressed protein to the desired site of action. Examples of signal peptides include those natively linked to the plant pathogenesis-related proteins, e.g. PR-1, PR-2, and the like. *See, e.g., Payne et al.,*  
10           1988. Examples of transit peptides include the chloroplast transit peptides such as those described in Von Heijne *et al.* (1991), Mazur *et al.* (1987), and Vorst *et al.* (1988); and mitochondrial transit peptides such as those described in Boutry *et al.* (1987). Also included are sequences that result in localization of the encoded protein to various cellular  
15           compartments such as the vacuole. *See, for example, Neuhaus et al.* (1991) and Chrispeels (1991).

          The chimeric DNA construct(s) of the invention may contain multiple copies of a promoter or multiple copies of a *NIM1* homologue coding sequence of the present  
20           invention. In addition, the construct(s) may include coding sequences for markers and coding sequences for other peptides such as signal or transit peptides, each in proper reading frame with the other functional elements in the DNA molecule. The preparation of  
25           such constructs are within the ordinary level of skill in the art.

          Useful markers include peptides providing herbicide, antibiotic or drug resistance, such as, for example, resistance to protoporphyrinogen oxidase inhibitors, hygromycin, kanamycin, G418, gentamycin, lincomycin, methotrexate, glyphosate, phosphinothricin, or  
30           the like. These markers can be used to select cells transformed with the chimeric DNA constructs of the invention from untransformed cells. Other useful markers are peptidic enzymes which can be easily detected by a visible reaction, for example a color reaction, for example luciferase,  $\beta$ -glucuronidase, or  $\beta$ -galactosidase.  
35           

          Chimeric genes designed for plant expression such as those described herein can be introduced into the plant cell in a number of art-recognized ways. Those skilled in the art will appreciate that the choice of method might depend on the type of plant (i.e. monocot or dicot) and/or organelle (i.e. nucleus, chloroplast, mitochondria) targeted for transformation.  
40           Suitable methods of transforming plant cells include microinjection (Crossway *et al.*, 1986), electroporation (Riggs *et al.*, 1986), *Agrobacterium* mediated transformation (Hinchee *et al.*, 1988; Ishida *et al.*, 1996), direct gene transfer (Paszowski *et al.*, 1984; Hayashimoto *et al.*, 1990), and ballistic particle acceleration using devices available from Agracetus, Inc.,  
45           Madison, Wisconsin and Dupont, Inc., Wilmington, Delaware (*see, for example, U.S. Patent 4,945,050; and McCabe et al.*, 1988). *See also, Weissinger et al.* (1988); Sanford *et al.* (1987) (onion); Christou *et al.* (1988) (soybean); McCabe *et al.* (1988) (soybean); Datta *et*  
50

5 *al.* (1990) (rice); Klein *et al.* (1988) (maize); Klein *et al.* (1988) (maize); Klein *et al.* (1988)  
(maize); Fromm *et al.* (1990); and Gordon-Kamm *et al.* (1990) (maize); Svab *et al.* (1990)  
(tobacco chloroplasts); Gordon-Kamm *et al.* (1993) (maize); Shimamoto *et al.* (1989) (rice);  
10 Christou *et al.* (1991) (rice); Datta *et al.* (1990) (rice); European Patent Application EP 0  
332 581 (orchardgrass and other *Pooideae*); Vasil *et al.* (1993) (wheat); Weeks *et al.* (1993)  
(wheat); Wan *et al.* (1994) (barley); Jahne *et al.* (1994) (barley); Umbeck *et al.* (1987)  
(cotton); Casas *et al.* (1993) (sorghum); Somers *et al.* (1992) (oats); Torbert *et al.* (1995)  
15 (oats); Weeks *et al.*, (1993) (wheat); WO 94/13822 (wheat); and Nehra *et al.* (1994) (wheat).  
A particularly preferred set of embodiments for the introduction of recombinant DNA  
molecules into maize by microprojectile bombardment can be found in Koziel *et al.* (1993);  
Hill *et al.* (1995) and Koziel *et al.* (1996). An additional preferred embodiment is the  
20 protoplast transformation method for maize as disclosed in EP 0 292 435.

Once a chimeric gene comprising a *NIM1* homologue coding sequence has been  
transformed into a particular plant species, it may be propagated in that species or moved  
into other varieties of the same species, particularly including commercial varieties, using  
25 traditional breeding techniques. Particularly preferred plants of the invention include the  
agronomically important crops listed above. The genetic properties engineered into the  
transgenic seeds and plants described above are passed on by sexual reproduction and  
can thus be maintained and propagated in progeny plants.



## EXAMPLES

The invention is illustrated in further detail by the following detailed procedures, preparations, and examples. The examples are for illustration only, and are not to be construed as limiting the scope of the present invention. Standard recombinant DNA and molecular cloning techniques used here are well known in the art and are described by Sambrook, *et al.*, 1989; by T.J. Silhavy, M.L. Berman, and L.W. Enquist, 1984; and by Ausubel, F.M. *et al.*, 1987.

I. Isolation of Homologues of the *Arabidopsis NIM1* GeneExample 1: Isolation of a *NIM1* Homologue from *Nicotiana tabacum*

Plasmid DNA from a mass excision of phage from a tobacco cDNA library is used as a template for PCR using the following primer pairs: 5'-AGATTATTGTCAAGTCTAATG-3' (SEQ ID NO:9) + 5'-TTCCATGTACCTTTGCTTC-3' (SEQ ID NO:10), and 5'-GCGGATCCATGGATAATAGTAGG-3' (SEQ ID NO:11) + 5'-GCGGATCCTATTTCCTAAAAGGG-3' (SEQ ID NO:12). Cycling conditions are preferably 94 degrees for one minute, 40 degrees for one minute, and 72 degrees for 1.5 minutes, and the reaction is preferably carried out for 40 cycles. PCR products are run out on agarose gels, excised, and cloned into pCRII-TOPO (Invitrogen).

The full-length cDNA sequence of this tobacco *NIM1* homologue is shown in SEQ ID NO:1, and the protein encoded by this cDNA sequence is shown in SEQ ID NO:2. A tobacco *NIM1* homologue comprising SEQ ID NO:1 has been deposited as pNOV1206 with the NRRL (Agricultural Research Service, Patent Culture Collection, Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A) on August 17, 1998, and assigned accession no. NRRL B-30051.

Example 2: Isolation of a *NIM1* Homologue from *Lycopersicon esculentum*

Phagemids are excised from  $\lambda$  ZAPII cDNA libraries of tomato using a protocol from Stratagene. Phagemids (plasmids) are mass-transformed into *E. coli* XL1-Blue in 10 pools of about 80,000 clones each and DNA is extracted from these pools. The pools are screened by PCR for the presence of *NIM1* homologues by PCR using the following

5 primers: 5'-AGATTATTGTCAAGTCTAATG-3' (SEQ ID NO:9) and 5'-  
TTCCATGTACCTTTGCTTC-3' (SEQ ID NO:10).

10 Sequences amplified from the pools are confirmed to contain *NIM1* homologues by  
cloning the PCR-amplified DNA fragment and sequencing. Pools are made successively  
smaller and screened by PCR using the same primers mentioned above for the presence of  
the *NIM1* homologues until a single clone containing the homologue is obtained. In the  
event that the cDNA clone contains a partial gene missing the 5' end, 5' RACE (Rapid  
Amplification of cDNA Ends) is used to obtain the full-length sequence of the gene.

15 The full-length cDNA sequence of this tomato *NIM1* homologue is shown in SEQ ID  
NO:3, and the protein encoded by this cDNA sequence is shown in SEQ ID NO:4. A tomato  
*NIM1* homologue comprising SEQ ID NO:3 has been deposited as pNOV1204 with the  
NRRL (Agricultural Research Service, Patent Culture Collection, Northern Regional  
20 Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A) on August 17,  
1998, and assigned accession no. NRRL B-30050.

25 Example 3: Isolation of a *NIM1* Homologue from *Brassica napus*

Phagemids are excised from  $\lambda$  ZAPII cDNA libraries of *Brassica napus* using a  
protocol from Stratagene. Phagemids (plasmids) are mass-transformed into *E. coli* XL1-  
30 Blue in 10 pools of about 80,000 clones each and DNA is extracted from these pools. The  
pools are screened by PCR for the presence of *NIM1* homologues by PCR using the  
following primers: 5'-AGATTATTGTCAAGTCTAATG-3' (SEQ ID NO:9) and 5'-  
TTCCATGTACCTTTGCTTC-3' (SEQ ID NO:10).

35 Sequences amplified from the pools are confirmed to contain *NIM1* homologues by  
cloning the PCR-amplified DNA fragment and sequencing. Pools are made successively  
smaller and screened by PCR using the same primers mentioned above for the presence of  
the *NIM1* homologues until a single clone containing the homologue is obtained. In the  
event that the cDNA clone contains a partial gene, missing the 5' end, 5' RACE (Rapid  
40 Amplification of cDNA Ends) is used to obtain the full-length sequence of the gene.

A partial cDNA sequence of this *Brassica napus* *NIM1* homologue is shown in SEQ  
45 ID NO:5, and the protein encoded by this cDNA sequence is shown in SEQ ID NO:6. A  
*Brassica napus* *NIM1* homologue comprising SEQ ID NO:5 has been deposited as  
pNOV1203 with the NRRL (Agricultural Research Service, Patent Culture Collection,

Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A) on August 17, 1998, and assigned accession no. NRRL B-30049.

#### Example 4: Isolation of a *NIM1* Homologue from *Arabidopsis thaliana*

BLAST searches using the *Arabidopsis* or tomato *NIM1* amino acid sequences as queries detect GenBank entry B26306, which contains *Arabidopsis* genomic sequence from the Bacterial Artificial Chromosome (BAC) F18D8. Part of the BAC sequence is predicted to encode a protein with significant similarity (47% amino acid identity) to *NIM1*. The following primers are designed to regions of the F18D8 sequence: 5'-TCAAGGCCTTGGATTCAGATG-3' (SEQ ID NO:13) and 5'-ATTAAGTGCCTACGTCCGTC-3' (SEQ ID NO:14).

The primers are used in a PCR reaction with DNA from a pFL61-based *Arabidopsis* cDNA library as a template. Preferable cycling conditions are 94 degrees for 30 seconds, 53 degrees for 30 seconds, 72 degrees for 30 seconds. The reaction is preferably run for 40 cycles. A PCR product of the predicted size (290 base pairs) is detected, and the cDNA clone corresponding to the F18D8 primers is purified from the cDNA library by sequential purification by passage of increasingly smaller amounts of the library through *E. coli* and re-diagnosis of the presence of the clone by PCR. Ultimately, a single positive clone is obtained and sequenced. The sequence of the clone confirms the presence of an open reading frame with significant homology to *NIM1*.

A full-length cDNA sequence of this *Arabidopsis thaliana NIM1* homologue is shown in SEQ ID NO:7, and the protein encoded by this cDNA sequence is shown in SEQ ID NO:8. An *Arabidopsis thaliana NIM1* homologue comprising SEQ ID NO:7 has been deposited as *AtNMLc5* in *E. coli* with the NRRL (Agricultural Research Service, Patent Culture Collection, Northern Regional Research Center, 1815 North University Street, Peoria, Illinois 61604, U.S.A) on May 25, 1999, and assigned accession no. NRRL B-30139.

#### Example 5: Design of Degenerate Primers

In addition to the *NIM1* gene (Ryals *et al.*, 1997) and the *NIM*-like gene described above in Example 4 (*AtNMLc5* - SEQ ID NO:7), *Arabidopsis thaliana* contains three other *NIM*-like (*NML*) genomic sequences: *AtNMLc2* (SEQ ID NO:15), *AtNMLc4-1* (SEQ ID

NO:17), and *AtNMLc4-2* (SEQ ID NO:19), where *c[#]* stands for the chromosome number on which the particular *NML* gene is located. Using the GCG Seqweb multiple sequence alignment program (Pretty, Wisconsin Genetics Computer Group), the *NIM1* sequences from *Arabidopsis thaliana* (Ryals *et al.*, 1997), *Nicotiana tabacum* (Example 1 - SEQ ID NO:1), and *Lycopersicon esculentum* (Example 2 - SEQ ID NO:3), as well as the *NML* sequences from *Arabidopsis thaliana* (SEQ ID NO:7, 15, 17, and 19) are aligned. Based on this alignment, three regions emerge with sufficient conservation to design degenerate PCR primers for PCR amplification of *NIM1* homologues from other crop species, including sugarbeet, sunflower, potato, and canola. The primers designed from these conserved regions are listed below in Table 1. The NIM 1(A-D) primers are designed using a lineup with only the *NIM1* genes from *Arabidopsis thaliana* (Ryals *et al.*, 1997), *Nicotiana tabacum* (Example 1 - SEQ ID NO:1), and *Lycopersicon esculentum* (Example 2 - SEQ ID NO:3). The NIM 2(A-D) primers are designed using a lineup with these three sequences in addition to the four *NML* sequences from *Arabidopsis thaliana* (SEQ ID NO:7, 15, 17, and 19). Primers are preferably synthesized by Genosys Biotechnologies, Inc. (The Woodlands, Texas). Positions of degeneracy are indicated in Table 1 by the notation of more than one base at a single site in the oligonucleotide. "Orientation" designates whether the primer is directed towards the 3' end (Downstream) or the 5' end (Upstream) of the cDNA.

Table 1: Degenerate Primers

Primer	Sequence (5' to 3')	SEQ ID NO:	Orientation
NIM 1A	GAGATTATTGTCAAGTCTAATGTAGATA T T	SEQ ID NO:21	Downstream
NIM 1B	ACTGGACTCGGATGATATTGAATTA T T T T G G	SEQ ID NO:22	Downstream
NIM 1C	TAAC TCAACATCATCAGAATCAAATGC T T C G C G	SEQ ID NO:23	Upstream
NIM 1D	GTTGAGCAAGAGCAACTCTATTTTCAAG T C CC G T	SEQ ID NO:24	Upstream
NIM 2A	TGCATAGAAATAATTGTGAAGTCTAATGTAGA T G TG C G T	SEQ ID NO:25	Downstream
NIM 2B	GGCACTGGACTCAGATGATGTTGAACT T T T GT	SEQ ID NO:26	Downstream
NIM 2C	AACTCAACATCATCAGAATCCAATGCC GT T G G	SEQ ID NO:27	Upstream
NIM 2D	AGTTGAGCAAGGCCAACTCGATTTTCAAAAT T C A T GG T	SEQ ID NO:28	Upstream

### Example 6: PCR Amplification of *NIM*-like DNA Fragments From Crop Species

*NIM*-like DNA fragments are amplified from *Arabidopsis*, tomato, tobacco, sugarbeet, sunflower, potato, and canola, using either genomic DNA or cDNA as templates. The primer combinations used, along with the expected fragment sizes, are listed below in Table 2.

Table 2: Primer combinations and DNA fragment sizes

Left Primer	Right Primer	Fragment Size (bp)
NIM 1A	NIM 1D	669
NIM 1A	NIM 1C	195
NIM 1B	NIM 1D	499
NIM 2A	NIM 2D	676
NIM 2A	NIM 2C	200
NIM 2B	NIM 2D	503

Degenerate primer PCR is preferably performed with Ready-To-Go PCR Beads (Amersham, Piscataway, NJ) in a GeneAmp PCR System 9700 (PE Applied Biosystems, Foster City, CA). 20 to 40 ng of genomic DNA or 5 to 10 ng of cDNA is used in each reaction, with each primer at a final concentration of 0.8  $\mu$ M. Preferable cycling parameters are as follows: 94°C for 1 minute; 3 cycles of [94°C for 30 seconds; 37°C for 30 seconds; 72°C for 2 minutes]; 35 cycles of [94°C for 30 seconds; 60°C for 30 seconds; 72°C for 2 minutes]; 72°C for 7 minutes; 4°C hold. Reaction products are analyzed on 2% agarose gels and DNA fragments of the appropriate size are excised. DNA fragments are isolated from agarose bands using, for example, the GeneClean III Kit (BIO 101, Inc., Carlsbad, CA) and cloned using, for example, the TOPO TA Cloning Kit (Invitrogen Corporation, Carlsbad, CA). Plasmids are isolated using, for example, the CONCERT Rapid Plasmid Miniprep System (Life Technologies, Inc., Rockville, MD) and sequenced by standard protocols.

*NIM*-like DNA fragments are obtained from all plant species attempted, and in many cases multiple, unique *NIM*-like sequences are isolated. Table 3 and Figure 2 detail the *NIM*-like fragments that are isolated.

Table 3: *NIM*-like PCR fragments

Species	Successful Primer Pairs	PCR Template	Unique Clones	SEQ ID NO:
<i>Arabidopsis</i>	1A/1D; 1B/1D	Genomic DNA	One	
Tobacco	1A/1D; 1B/1D; 2A/2D; 2B/2D	cDNA	Four	SEQ ID NO: 29, 31, 33, and 35
Tomato	1A/1D; 1B/1D; 2A/2D; 2B/2D	Genomic DNA, cDNA	One	SEQ ID NO: 37
Sugarbeet	1B/1D; 2B/2D	Genomic DNA, cDNA	One	SEQ ID NO: 39
Sunflower	2B/2D	cDNA	Two	SEQ ID NO: 41 and 43
Potato	1A/1D; 1A/1C; 1B/1D; 2A/2D; 2B/2D	cDNA	Three	SEQ ID NO: 45, 47, and 49
Canola	2B/2D	cDNA	Four	SEQ ID NO: 51, 53, 55, and 57

Based on these results, the degenerate primer PCR described above can amplify *NIM*-like fragments from a wide variety of plant species. In particular, the primer combination of NIM 2B/NIM 2D is successful with cDNA as a template from all species attempted. The use of Ready-To-Go PCR Beads is especially preferably for obtaining products. In addition, using cDNA as a template is preferable for all samples except *Arabidopsis*, tomato and sugarbeet, where genomic DNA is sufficient.

#### Example 7: Additional Degenerate Primers

A new pair of degenerate primers is designed based on a sequence alignment of the four tobacco fragments (SEQ ID NO: 29, 31, 33, and 35) and the tomato sequence (SEQ ID NO: 37) for use in determining whether tomato also contains similar *NIM*-like sequences that are not amplified with the degenerate primers listed in Table 1. The primers designed from these fragments are listed below in Table 3 and are preferably synthesized by Genosys Biotechnologies, Inc. (The Woodlands, Texas). Positions of degeneracy are indicated in Table 3 by the notation of more than one base at a single site in the

oligonucleotide. "Orientation" designates whether the primer is directed towards the 3' end (Downstream) or the 5' end (Upstream) of the cDNA.

Table 4: Additional degenerate primers

Primer	Sequence (5' TO 3')	SEQ ID NO:	Orientation
NIM 3A	TAGATGAAGCATACGCTCTCCACTATGCTGT T C T T T	SEQ ID NO:59	Downstream
NIM 3B	GGCTCCTTACGCATGGCAGCAACATGAAGGAC T C T TG C	SEQ ID NO:60	Upstream

Degenerate primer PCR is performed as described above using tomato cDNA, and potential products are cloned and sequenced. The sequence analysis reveals two classes of *NIM*-like fragments: the first is identical to the tomato sequence shown in SEQ ID NO: 37, and the second is unique in tomato and 88% identical to the tobacco sequences shown in SEQ ID NO:31 and 33. The sequence of this new tomato sequence is presented in SEQ ID NO:61.

#### Example 8: Full-length *NIM*-like cDNA's

Corresponding cDNA sequences upstream and downstream from *NIM*-like PCR fragments are preferably obtained by RACE PCR using the SMART RACE cDNA Amplification Kit (Clontech, Palo Alto, CA). Preferably, at least three independent RACE products are sequenced for each 5'- or 3'-end in order to eliminate PCR errors. Resulting full-length cDNA sequences for Sugarbeet, Sunflower B, and Tobacco B *NIM1* homologues, which correspond to the *NIM*-like PCR fragments shown in SEQ ID NO:39, 43, and 31 are presented as SEQ ID NO:63, 65, and 73 respectively.

*NIM*-like *Arabidopsis thaliana* cDNA's corresponding to the *NIM*-like genomic sequences *AtNMLc2* (SEQ ID NO:15), *AtNMLc4-1* (SEQ ID NO:17), and *AtNMLc4-2* (SEQ ID NO:19), are preferably cloned by RT-PCR. Total RNA from *Arabidopsis thaliana* is reverse transcribed using oligo dT primer. The resulting first strand cDNA is amplified by PCR using specific sense and antisense oligonucleotide primers designed based on the 5' and 3' ends of the coding region of each genomic sequence (SEQ ID NO:15, 17, and 19). PCR fragments of the predicted sizes are cloned into a vector and sequenced to confirm that these cDNA clones correspond to the *NIM*-like genomic sequences. A cDNA sequence corresponding to the *NIM*-like genomic sequence *AtNMLc2* (SEQ ID NO:15) is presented as

5 SEQ ID NO:67; a full-length cDNA sequence corresponding to the *NIM*-like genomic  
sequence *AtNMLc4-1* (SEQ ID NO:17) is presented as SEQ ID NO:69; and a full-length  
cDNA sequence corresponding to the *NIM*-like genomic sequence *AtNMLc4-2* (SEQ ID  
10 NO:19) is presented as SEQ ID NO:71.

#### Example 9: Northern Analysis

15 Northern data shows that expression of the sugarbeet *NIM*-like clone (SEQ ID NO:39  
and 63) increases three to seven fold after 100µM or 300 µM BTH (benzo(1,2,3)thiadiazole-  
7-carbothioic acid *S*-methyl ester) treatment. Also, Northern data shows that expression of  
the Sunflower A *NIM*-like clone (SEQ ID NO:41) is constitutive. Furthermore, Northern data  
20 shows that expression of the Sunflower B *NIM*-like clone (SEQ ID NO:43 and 65) increases  
two fold after 100µM or 300 µM BTH treatment.

#### II. Expression of the Gene Sequences of the Invention In Plants

25 A *NIM1* homologue of the present invention can be incorporated into plant cells using  
conventional recombinant DNA technology. Generally, this involves inserting a coding  
sequence of the invention into an expression system to which the coding sequence is  
heterologous (i.e., not normally present) using standard cloning procedures known in the  
30 art. The vector contains the necessary elements for the transcription and translation of the  
inserted protein-coding sequences. A large number of vector systems known in the art can  
be used, such as plasmids, bacteriophage viruses and other modified viruses. Suitable  
35 vectors include, but are not limited to, viral vectors such as lambda vector systems λgt11,  
λgt10 and Charon 4; plasmid vectors such as pBI121, pBR322, pACYC177, pACYC184,  
pAR series, pKK223-3, pUC8, pUC9, pUC18, pUC19, pLG339, pRK290, pKC37,  
40 pKC101, pCDNAII; and other similar systems. The components of the expression system  
may also be modified to increase expression. For example, truncated sequences,  
nucleotide substitutions or other modifications may be employed. The expression systems  
described herein can be used to transform virtually any crop plant cell under suitable  
45 conditions. Transformed cells can be regenerated into whole plants such that the *NIM1*  
homologue increases SAR gene expression and enhances disease resistance in the  
transgenic plants.



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### Example 10: Construction of Plant Expression Cassettes

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Coding sequences intended for expression in transgenic plants are first assembled in expression cassettes behind a suitable promoter expressible in plants. The expression cassettes may also comprise any further sequences required or selected for the expression of the transgene. Such sequences include, but are not restricted to, transcription terminators, extraneous sequences to enhance expression such as introns, vital sequences, and sequences intended for the targeting of the gene product to specific organelles and cell compartments. These expression cassettes can then be easily transferred to the plant transformation vectors described below. The following is a description of various components of typical expression cassettes.

#### 1. Promoters

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The selection of the promoter used in expression cassettes will determine the spatial and temporal expression pattern of the transgene in the transgenic plant. Selected promoters will express transgenes in specific cell types (such as leaf epidermal cells, mesophyll cells, root cortex cells) or in specific tissues or organs (roots, leaves or flowers, for example) and the selection will reflect the desired location of accumulation of the gene product. Alternatively, the selected promoter may drive expression of the gene under various inducing conditions. Promoters vary in their strength, i.e., ability to promote transcription. Depending upon the host cell system utilized, any one of a number of suitable promoters can be used, including the gene's native promoter. The following are non-limiting examples of promoters that may be used in expression cassettes.

#### a. Constitutive Expression, the Ubiquitin Promoter:

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Ubiquitin is a gene product known to accumulate in many cell types and its promoter has been cloned from several species for use in transgenic plants (e.g. sunflower - Binet *et al.*, 1991; maize - Christensen *et al.*, 1989; and *Arabidopsis* - Norris *et al.*, 1993). The maize ubiquitin promoter has been developed in transgenic monocot systems and its sequence and vectors constructed for monocot transformation are disclosed in the patent publication EP 0 342 926 (to Lubrizol). Taylor *et al.* (1993) describe a vector (pAHC25) that comprises the maize ubiquitin promoter and first intron and its high activity in cell suspensions of numerous monocotyledons when introduced via microprojectile

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5 bombardment. The *Arabidopsis* ubiquitin promoter is especially preferred for use with the  
10 *NIM1* homologues of the present invention. The ubiquitin promoter is suitable for gene  
expression in transgenic plants, both monocotyledons and dicotyledons. Suitable vectors  
are derivatives of pAHC25 or any of the transformation vectors described in this application,  
15 modified by the introduction of the appropriate ubiquitin promoter and/or intron sequences.

b. Constitutive Expression, the CaMV 35S Promoter:

15 Construction of the plasmid pCGN1761 is described in the published patent  
application EP 0 392 225 (Example 23). pCGN1761 contains the "double" CaMV 35S  
promoter and the *tm1* transcriptional terminator with a unique *EcoRI* site between the  
promoter and the terminator and has a pUC-type backbone. A derivative of pCGN1761 is  
20 constructed which has a modified polylinker which includes *NotI* and *XhoI* sites in addition  
to the existing *EcoRI* site. This derivative is designated pCGN1761ENX. pCGN1761ENX is  
useful for the cloning of cDNA sequences or coding sequences (including microbial ORF  
sequences) within its polylinker for the purpose of their expression under the control of the  
25 35S promoter in transgenic plants. The entire 35S promoter-coding sequence-*tm1*  
terminator cassette of such a construction can be excised by *HindIII*, *SphI*, *Sall*, and *XbaI*  
sites 5' to the promoter and *XbaI*, *BamHI* and *BglII* sites 3' to the terminator for transfer to  
transformation vectors such as those described below. Furthermore, the double 35S  
30 promoter fragment can be removed by 5' excision with *HindIII*, *SphI*, *Sall*, *XbaI*, or *PstI*, and  
3' excision with any of the polylinker restriction sites (*EcoRI*, *NotI* or *XhoI*) for replacement  
with another promoter. If desired, modifications around the cloning sites can be made by  
the introduction of sequences that may enhance translation. This is particularly useful when  
35 overexpression is desired. For example, pCGN1761ENX may be modified by optimization  
of the translational initiation site as described in Example 37 of U.S. Patent No. 5,639,949.

40 c. Constitutive Expression, the Actin Promoter:

Several isoforms of actin are known to be expressed in most cell types and  
consequently the actin promoter is a good choice for a constitutive promoter. In particular,  
45 the promoter from the rice *Act1* gene has been cloned and characterized (McElroy *et al.*,  
1990). A 1.3kb fragment of the promoter was found to contain all the regulatory elements  
required for expression in rice protoplasts. Furthermore, numerous expression vectors  
based on the *Act1* promoter have been constructed specifically for use in monocotyledons  
50 (McElroy *et al.*, 1991). These incorporate the *Act1*-intron 1, *Adh1* 5' flanking sequence and

5 *Adhl*-intron 1 (from the maize alcohol dehydrogenase gene) and sequence from the CaMV  
35S promoter. Vectors showing highest expression were fusions of 35S and *Act1* intron or  
the *Act1* 5' flanking sequence and the *Act1* intron. Optimization of sequences around the  
10 initiating ATG (of the GUS reporter gene) also enhanced expression. The promoter  
expression cassettes described by McElroy *et al.* (1991) can be easily modified for gene  
expression and are particularly suitable for use in monocotyledonous hosts. For example,  
promoter-containing fragments is removed from the McElroy constructions and used to  
15 replace the double 35S promoter in pCGN1761ENX, which is then available for the insertion  
of specific gene sequences. The fusion genes thus constructed can then be transferred to  
appropriate transformation vectors. In a separate report, the rice *Act1* promoter with its first  
intron has also been found to direct high expression in cultured barley cells (Chibbar *et al.*,  
20 1993).

d. Inducible Expression, the PR-1 Promoter:

25 The double 35S promoter in pCGN1761ENX may be replaced with any other promoter  
of choice that will result in suitably high expression levels. By way of example, one of the  
chemically regulatable promoters described in U.S. Patent No. 5,614,395 may replace the  
double 35S promoter. The promoter of choice is preferably excised from its source by  
30 restriction enzymes, but can alternatively be PCR-amplified using primers that carry  
appropriate terminal restriction sites. Should PCR-amplification be undertaken, then the  
promoter should be re-sequenced to check for amplification errors after the cloning of the  
amplified promoter in the target vector. The chemically/pathogen regulatable tobacco PR-  
1a promoter is cleaved from plasmid pCIB1004 (for construction, see example 21 of  
35 EP 0 332 104) and transferred to plasmid pCGN1761ENX (Uknes *et al.*, 1992). pCIB1004  
is cleaved with *NcoI* and the resultant 3' overhang of the linearized fragment is rendered  
blunt by treatment with T4 DNA polymerase. The fragment is then cleaved with *HindIII* and  
40 the resultant PR-1a promoter-containing fragment is gel purified and cloned into  
pCGN1761ENX from which the double 35S promoter has been removed. This is done by  
cleavage with *XhoI* and blunting with T4 polymerase, followed by cleavage with *HindIII* and  
isolation of the larger vector-terminator containing fragment into which the pCIB1004  
45 promoter fragment is cloned. This generates a pCGN1761ENX derivative with the PR-1a  
promoter and the *tm1* terminator and an intervening polylinker with unique *EcoRI* and *NotI*  
sites. The selected coding sequence can be inserted into this vector, and the fusion  
products (*i.e.* promoter-gene-terminator) can subsequently be transferred to any selected  
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5 transformation vector, including those described *infra*. Various chemical regulators may be employed to induce expression of the selected coding sequence in the plants transformed according to the present invention, including the benzothiadiazole, isonicotinic acid, and  
10 salicylic acid compounds disclosed in U.S. Patent Nos. 5,523,311 and 5,614,395.

e. Inducible Expression, an Ethanol-Inducible Promoter:

15 A promoter inducible by certain alcohols or ketones, such as ethanol, may also be used to confer inducible expression of a coding sequence of the present invention. Such a promoter is for example the *alcA* gene promoter from *Aspergillus nidulans* (Caddick *et al.*, 1998). In *A. nidulans*, the *alcA* gene encodes alcohol dehydrogenase I, the expression of which is regulated by the AlcR transcription factors in presence of the chemical inducer.  
20 For the purposes of the present invention, the CAT coding sequences in plasmid p*alcA*:CAT comprising a *alcA* gene promoter sequence fused to a minimal 35S promoter (Caddick *et al.*, 1998) are replaced by a coding sequence of the present invention to form an expression cassette having the coding sequence under the control of the *alcA* gene promoter. This is  
25 carried out using methods well known in the art.

f. Inducible Expression, a Glucocorticoid-Inducible Promoter:

30 Induction of expression of a NIM1 homologue of the present invention using systems based on steroid hormones is also contemplated. For example, a glucocorticoid-mediated induction system is used (Aoyama and Chua, 1997) and gene expression is induced by application of a glucocorticoid, for example a synthetic glucocorticoid, preferably  
35 dexamethasone, preferably at a concentration ranging from 0.1mM to 1mM, more preferably from 10mM to 100mM. For the purposes of the present invention, the luciferase gene sequences are replaced by a gene sequence encoding a NIM1 homologue to form an expression cassette having the gene sequence encoding a NIM1 homologue under the  
40 control of six copies of the GAL4 upstream activating sequences fused to the 35S minimal promoter. This is carried out using methods well known in the art. The trans-acting factor comprises the GAL4 DNA-binding domain (Keegan *et al.*, 1986) fused to the transactivating domain of the herpes viral protein VP16 (Triezenberg *et al.*, 1988) fused to the hormone-  
45 binding domain of the rat glucocorticoid receptor (Picard *et al.*, 1988). The expression of the fusion protein is controlled by any promoter suitable for expression in plants known in the art or described here. This expression cassette is also comprised in the plant comprising  
50 the gene sequence encoding a NIM1 homologue fused to the 6xGAL4/minimal promoter.

Thus, tissue- or organ-specificity of the fusion protein is achieved leading to inducible tissue- or organ-specificity of the NIM1 homologue.

g. Root Specific Expression:

Another pattern of gene expression is root expression. A suitable root promoter is described by de Framond (1991) and also in the published patent application EP 0 452 269. This promoter is transferred to a suitable vector such as pCGN1761ENX for the insertion of a selected gene and subsequent transfer of the entire promoter-gene-terminator cassette to a transformation vector of interest.

h. Wound-Inducible Promoters:

Wound-inducible promoters may also be suitable for gene expression. Numerous such promoters have been described (e.g. Xu *et al.*, 1993; Logemann *et al.*, 1989; Rohrmeier & Lehle, 1993; Firek *et al.*, 1993; Warner *et al.*, 1993) and all are suitable for use with the instant invention. Logemann *et al.* describe the 5' upstream sequences of the dicotyledonous potato *wun1* gene. Xu *et al.* show that a wound-inducible promoter from the dicotyledon potato (*pin2*) is active in the monocotyledon rice. Further, Rohrmeier & Lehle describe the cloning of the maize *Wip1* cDNA which is wound induced and which can be used to isolate the cognate promoter using standard techniques. Similar, Firek *et al.* and Warner *et al.* have described a wound-induced gene from the monocotyledon *Asparagus officinalis*, which is expressed at local wound and pathogen invasion sites. Using cloning techniques well known in the art, these promoters can be transferred to suitable vectors, fused to the genes pertaining to this invention, and used to express these genes at the sites of plant wounding.

i. Pith-Preferred Expression:

Patent Application WO 93/07278 describes the isolation of the maize *trpA* gene, which is preferentially expressed in pith cells. The gene sequence and promoter extending up to -1726 bp from the start of transcription are presented. Using standard molecular biological techniques, this promoter, or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a foreign gene in a pith-preferred manner. In fact, fragments containing the pith-preferred promoter or parts thereof can be transferred to any vector and modified for utility in transgenic plants.

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j. Leaf-Specific Expression:

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A maize gene encoding phosphoenol carboxylase (PEPC) has been described by Hudspeth & Grula (1989). Using standard molecular biological techniques the promoter for this gene can be used to drive the expression of any gene in a leaf-specific manner in transgenic plants.

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k. Pollen-Specific Expression:

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WO 93/07278 describes the isolation of the maize calcium-dependent protein kinase (CDPK) gene which is expressed in pollen cells. The gene sequence and promoter extend up to 1400 bp from the start of transcription. Using standard molecular biological techniques, this promoter or parts thereof, can be transferred to a vector such as pCGN1761 where it can replace the 35S promoter and be used to drive the expression of a NIM1 homologue of the present invention in a pollen-specific manner.

25

2. Transcriptional Terminators

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A variety of transcriptional terminators are available for use in expression cassettes. These are responsible for the termination of transcription beyond the transgene and its correct polyadenylation. Appropriate transcriptional terminators are those that are known to function in plants and include the CaMV 35S terminator, the *tm1* terminator, the nopaline synthase terminator and the pea *rbcS* E9 terminator. These can be used in both monocotyledons and dicotyledons. In addition, a gene's native transcription terminator may be used.

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3. Sequences for the Enhancement or Regulation of Expression

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Numerous sequences have been found to enhance gene expression from within the transcriptional unit and these sequences can be used in conjunction with the genes of this invention to increase their expression in transgenic plants.

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Various intron sequences have been shown to enhance expression, particularly in monocotyledonous cells. For example, the introns of the maize *Adhl* gene have been found to significantly enhance the expression of the wild-type gene under its cognate promoter when introduced into maize cells. Intron 1 was found to be particularly effective and enhanced expression in fusion constructs with the chloramphenicol acetyltransferase gene (Callis *et al.*, 1987). In the same experimental system, the intron from the maize *bronze1*

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5 gene had a similar effect in enhancing expression. Intron sequences have been routinely incorporated into plant transformation vectors, typically within the non-translated leader.

A number of non-translated leader sequences derived from viruses are also known to enhance expression, and these are particularly effective in dicotyledonous cells.  
10 Specifically, leader sequences from Tobacco Mosaic Virus (TMV, the "W-sequence"), Maize Chlorotic Mottle Virus (MCMV), and Alfalfa Mosaic Virus (AMV) have been shown to be effective in enhancing expression (*e.g.* Gallie *et al.*, 1987; Skuzeski *et al.*, 1990).

#### 15 4. Targeting of the Gene Product Within the Cell

Various mechanisms for targeting gene products are known to exist in plants and the sequences controlling the functioning of these mechanisms have been characterized in some detail. For example, the targeting of gene products to the chloroplast is controlled by  
20 a signal sequence found at the amino terminal end of various proteins which is cleaved during chloroplast import to yield the mature protein (*e.g.* Comai *et al.*, 1988). These signal sequences can be fused to heterologous gene products to effect the import of heterologous products into the chloroplast (van den Broeck, *et al.*, 1985). DNA encoding for appropriate  
25 signal sequences can be isolated from the 5' end of the cDNAs encoding the RUBISCO protein, the CAB protein, the EPSP synthase enzyme, the GS2 protein and many other proteins which are known to be chloroplast localized. *See also*, the section entitled  
30 "Expression With Chloroplast Targeting" in Example 37 of U.S. Patent No. 5,639,949.

Other gene products are localized to other organelles such as the mitochondrion and the peroxisome (*e.g.* Unger *et al.*, 1989). The cDNAs encoding these products can also be manipulated to effect the targeting of heterologous gene products to these organelles.  
35 Examples of such sequences are the nuclear-encoded ATPases and specific aspartate amino transferase isoforms for mitochondria. Targeting cellular protein bodies has been described by Rogers *et al.* (1985).

In addition, sequences have been characterized which cause the targeting of gene  
40 products to other cell compartments. Amino terminal sequences are responsible for targeting to the ER, the apoplast, and extracellular secretion from aleurone cells (Koehler & Ho, 1990). Additionally, amino terminal sequences in conjunction with carboxy terminal  
45 sequences are responsible for vacuolar targeting of gene products (Shinshi *et al.*, 1990).

By the fusion of the appropriate targeting sequences described above to transgene sequences of interest it is possible to direct the transgene product to any organelle or cell compartment. For chloroplast targeting, for example, the chloroplast signal sequence from  
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5 the RUBISCO gene, the CAB gene, the EPSP synthase gene, or the GS2 gene is fused in  
frame to the amino terminal ATG of the transgene. The signal sequence selected should  
include the known cleavage site, and the fusion constructed should take into account any  
10 amino acids after the cleavage site which are required for cleavage. In some cases this  
requirement may be fulfilled by the addition of a small number of amino acids between the  
cleavage site and the transgene ATG or, alternatively, replacement of some amino acids  
within the transgene sequence. Fusions constructed for chloroplast import can be tested  
15 for efficacy of chloroplast uptake by *in vitro* translation of *in vitro* transcribed constructions  
followed by *in vitro* chloroplast uptake using techniques described by Bartlett *et al.* (1982)  
and Wasmann *et al.* (1986). These construction techniques are well known in the art and  
are equally applicable to mitochondria and peroxisomes.

20 The above-described mechanisms for cellular targeting can be utilized not only in  
conjunction with their cognate promoters, but also in conjunction with heterologous  
promoters so as to effect a specific cell-targeting goal under the transcriptional regulation of  
a promoter that has an expression pattern different to that of the promoter from which the  
25 targeting signal derives.

#### Example 11: Construction of Plant Transformation Vectors

30 Numerous transformation vectors available for plant transformation are known to  
those of ordinary skill in the plant transformation arts, and the genes pertinent to this  
invention can be used in conjunction with any such vectors. The selection of vector will  
depend upon the preferred transformation technique and the target species for  
35 transformation. For certain target species, different antibiotic or herbicide selection markers  
may be preferred. Selection markers used routinely in transformation include the *nptII*  
gene, which confers resistance to kanamycin and related antibiotics (Messing & Vierra,  
1982; Bevan *et al.*, 1983), the *bar* gene, which confers resistance to the herbicide  
40 phosphinothricin (White *et al.*, 1990; Spencer *et al.*, 1990), the *hph* gene, which confers  
resistance to the antibiotic hygromycin (Blochinger & Diggelmann), and the *dhfr* gene, which  
confers resistance to methatrexate (Bourouis *et al.*, 1983), and the EPSPS gene, which  
45 confers resistance to glyphosate (U.S. Patent Nos. 4,940,935 and 5,188,642).

##### 1. Vectors Suitable for *Agrobacterium* Transformation



5 Many vectors are available for transformation using *Agrobacterium tumefaciens*. These typically carry at least one T-DNA border sequence and include vectors such as pBIN19 (Bevan, Nucl. Acids Res. (1984)) and pXYZ. Below, the construction of two typical  
10 vectors suitable for *Agrobacterium* transformation is described.

a. pCIB200 and pCIB2001:

15 The binary vectors pCIB200 and pCIB2001 are used for the construction of recombinant vectors for use with *Agrobacterium* and are constructed in the following manner. pTJS75kan is created by *NarI* digestion of pTJS75 (Schmidhauser & Helinski, 1985) allowing excision of the tetracycline-resistance gene, followed by insertion of an *AccI* fragment from pUC4K carrying an NPTII (Messing & Vierra, 1982; Bevan *et al.*, 1983; McBride *et al.*, 1990). *XhoI* linkers are ligated to the *EcoRV* fragment of PCIB7 which  
20 contains the left and right T-DNA borders, a plant selectable *nos/nptII* chimeric gene and the pUC polylinker (Rothstein *et al.*, 1987), and the *XhoI*-digested fragment are cloned into *Sall*-digested pTJS75kan to create pCIB200 (see also EP 0 332 104, example 19). pCIB200 contains the following unique polylinker restriction sites: *EcoRI*, *SstI*, *KpnI*, *BglII*, *XbaI*, and *Sall*. pCIB2001 is a derivative of pCIB200 created by the insertion into the polylinker of additional restriction sites. Unique restriction sites in the polylinker of  
30 pCIB2001 are *EcoRI*, *SstI*, *KpnI*, *BglII*, *XbaI*, *Sall*, *MluI*, *BclI*, *AvrII*, *ApaI*, *HpaI*, and *StuI*. pCIB2001, in addition to containing these unique restriction sites also has plant and bacterial kanamycin selection, left and right T-DNA borders for *Agrobacterium*-mediated transformation, the RK2-derived *trfA* function for mobilization between *E. coli* and other  
35 hosts, and the *OriT* and *OriV* functions also from RK2. The pCIB2001 polylinker is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

b. pCIB10 and Hygromycin Selection Derivatives thereof:

40 The binary vector pCIB10 contains a gene encoding kanamycin resistance for selection in plants and T-DNA right and left border sequences and incorporates sequences from the wide host-range plasmid pRK252 allowing it to replicate in both *E. coli* and *Agrobacterium*. Its construction is described by Rothstein *et al.* (1987). Various derivatives  
45 of pCIB10 are constructed which incorporate the gene for hygromycin B phosphotransferase described by Gritz *et al.*, 1983). These derivatives enable selection of transgenic plant cells on hygromycin only (pCIB743), or hygromycin and kanamycin (pCIB715, pCIB717).  
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## 2. Vectors Suitable for non-*Agrobacterium* Transformation

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Transformation without the use of *Agrobacterium tumefaciens* circumvents the requirement for T-DNA sequences in the chosen transformation vector and consequently vectors lacking these sequences can be utilized in addition to vectors such as the ones described above which contain T-DNA sequences. Transformation techniques that do not rely on *Agrobacterium* include transformation via particle bombardment, protoplast uptake (e.g. PEG and electroporation) and microinjection. The choice of vector depends largely on the preferred selection for the species being transformed. Below, the construction of typical vectors suitable for non-*Agrobacterium* transformation is described.

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### a. pCIB3064:

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pCIB3064 is a pUC-derived vector suitable for direct gene transfer techniques in combination with selection by the herbicide basta (or phosphinothricin). The plasmid pCIB246 comprises the CaMV 35S promoter in operational fusion to the *E. coli* GUS gene and the CaMV 35S transcriptional terminator and is described in the PCT published application WO 93/07278. The 35S promoter of this vector contains two ATG sequences 5' of the start site. These sites are mutated using standard PCR techniques in such a way as to remove the ATGs and generate the restriction sites *SspI* and *PvuII*. The new restriction sites are 96 and 37 bp away from the unique *Sall* site and 101 and 42 bp away from the actual start site. The resultant derivative of pCIB246 is designated pCIB3025. The GUS gene is then excised from pCIB3025 by digestion with *Sall* and *SacI*, the termini rendered blunt and religated to generate plasmid pCIB3060. The plasmid pJIT82 is obtained from the John Innes Centre, Norwich and the a 400 bp *SmaI* fragment containing the *bar* gene from *Streptomyces viridochromogenes* is excised and inserted into the *HpaI* site of pCIB3060 (Thompson *et al.*, 1987). This generated pCIB3064, which comprises the *bar* gene under the control of the CaMV 35S promoter and terminator for herbicide selection, a gene for ampicillin resistance (for selection in *E. coli*) and a polylinker with the unique sites *SphI*, *PstI*, *HindIII*, and *BamHI*. This vector is suitable for the cloning of plant expression cassettes containing their own regulatory signals.

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### b. pSOG19 and pSOG35:

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pSOG35 is a transformation vector that utilizes the *E. coli* gene dihydrofolate reductase (DFR) as a selectable marker conferring resistance to methotrexate. PCR is

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5 used to amplify the 35S promoter (-800 bp), intron 6 from the maize Adh1 gene (-550 bp)  
and 18 bp of the GUS untranslated leader sequence from pSOG10. A 250-bp fragment  
encoding the *E. coli* dihydrofolate reductase type II gene is also amplified by PCR and  
10 these two PCR fragments are assembled with a *SacI-PstI* fragment from pB1221 (Clontech)  
which comprises the pUC19 vector backbone and the nopaline synthase terminator.  
Assembly of these fragments generates pSOG19 which contains the 35S promoter in fusion  
with the intron 6 sequence, the GUS leader, the DHFR gene and the nopaline synthase  
terminator. Replacement of the GUS leader in pSOG19 with the leader sequence from  
15 Maize Chlorotic Mottle Virus (MCMV) generates the vector pSOG35. pSOG19 and pSOG35  
carry the pUC gene for ampicillin resistance and have *HindIII*, *SphI*, *PstI* and *EcoRI* sites  
available for the cloning of foreign substances.

#### 20 Example 12: Transformation

Once the gene sequence of interest has been cloned into an expression system, it is  
25 transformed into a plant cell. Methods for transformation and regeneration of plants are  
well known in the art. For example, Ti plasmid vectors have been utilized for the delivery of  
foreign DNA, as well as direct DNA uptake, liposomes, electroporation, micro-injection, and  
microprojectiles. In addition, bacteria from the genus *Agrobacterium* can be utilized to  
30 transform plant cells. Below are descriptions of representative techniques for transforming  
both dicotyledonous and monocotyledonous plants.

##### 35 1. Transformation of Dicotyledons

Transformation techniques for dicotyledons are well known in the art and include  
*Agrobacterium*-based techniques and techniques that do not require *Agrobacterium*. Non-  
*Agrobacterium* techniques involve the uptake of exogenous genetic material directly by  
40 protoplasts or cells. This can be accomplished by PEG or electroporation mediated uptake,  
particle bombardment-mediated delivery, or microinjection. Examples of these techniques  
are described by Paszkowski *et al.*, 1984; Potrykus *et al.*, 1985; Reich *et al.*, 1986; and  
Klein *et al.*, 1987. In each case the transformed cells are regenerated to whole plants using  
45 standard techniques known in the art.

*Agrobacterium*-mediated transformation is a preferred technique for transformation of  
dicotyledons because of its high efficiency of transformation and its broad utility with many  
50 different species. *Agrobacterium* transformation typically involves the transfer of the binary

5 vector carrying the foreign DNA of interest (e.g. pCIB200 or pCIB2001) to an appropriate  
*Agrobacterium* strain which may depend of the complement of *vir* genes carried by the host  
*Agrobacterium* strain either on a co-resident Ti plasmid or chromosomally (e.g. strain  
10 CIB542 for pCIB200 and pCIB2001 (Uknes *et al.*, 1993). The transfer of the recombinant  
binary vector to *Agrobacterium* is accomplished by a triparental mating procedure using *E.*  
*coli* carrying the recombinant binary vector, a helper *E. coli* strain which carries a plasmid  
such as pRK2013 and which is able to mobilize the recombinant binary vector to the target  
15 *Agrobacterium* strain. Alternatively, the recombinant binary vector can be transferred to  
*Agrobacterium* by DNA transformation (Höfgen & Willmitzer, 1988).

Transformation of the target plant species by recombinant *Agrobacterium* usually  
involves co-cultivation of the *Agrobacterium* with explants from the plant and follows  
20 protocols well known in the art. Transformed tissue is regenerated on selectable medium  
carrying the antibiotic or herbicide resistance marker present between the binary plasmid T-  
DNA borders.

Another approach to transforming plant cells with a gene involves propelling inert or  
25 biologically active particles at plant tissues and cells. This technique is disclosed in U.S.  
Patent Nos. 4,945,050, 5,036,006, and 5,100,792. Generally, this procedure involves  
propelling inert or biologically active particles at the cells under conditions effective to  
penetrate the outer surface of the cell and afford incorporation within the interior thereof.  
30 When inert particles are utilized, the vector can be introduced into the cell by coating the  
particles with the vector containing the desired gene. Alternatively, the target cell can be  
surrounded by the vector so that the vector is carried into the cell by the wake of the  
particle. Biologically active particles (e.g., dried yeast cells, dried bacterium or a  
35 bacteriophage, each containing DNA sought to be introduced) can also be propelled into  
plant cell tissue.

## 40 2. Transformation of Monocotyledons

Transformation of most monocotyledon species has now also become routine.  
Preferred techniques include direct gene transfer into protoplasts using PEG or  
electroporation techniques, and particle bombardment into callus tissue. Transformations  
45 can be undertaken with a single DNA species or multiple DNA species (*i.e.* co-  
transformation) and both these techniques are suitable for use with this invention. Co-  
transformation may have the advantage of avoiding complete vector construction and of  
generating transgenic plants with unlinked loci for the gene of interest and the selectable  
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5 marker, enabling the removal of the selectable marker in subsequent generations, should  
this be regarded desirable. However, a disadvantage of the use of co-transformation is the  
less than 100% frequency with which separate DNA species are integrated into the genome  
(Schocher *et al.*, 1986).

10 Patent Applications EP 0 292 435, EP 0 392 225, and WO 93/07278 describe  
techniques for the preparation of callus and protoplasts from an elite inbred line of maize,  
transformation of protoplasts using PEG or electroporation, and the regeneration of maize  
plants from transformed protoplasts. Gordon-Kamm *et al.* (1990) and Fromm *et al.* (1990)  
15 have published techniques for transformation of A188-derived maize line using particle  
bombardment. Furthermore, WO 93/07278 and Koziel *et al.* (1993) describe techniques for  
the transformation of elite inbred lines of maize by particle bombardment. This technique  
utilizes immature maize embryos of 1.5-2.5 mm length excised from a maize ear 14-15 days  
20 after pollination and a PDS-1000He Biolistics device for bombardment.

Transformation of rice can also be undertaken by direct gene transfer techniques  
utilizing protoplasts or particle bombardment. Protoplast-mediated transformation has been  
25 described for *Japonica*-types and *Indica*-types (Zhang *et al.*, 1988; Shimamoto *et al.*, 1989;  
Datta *et al.*, 1990). Both types are also routinely transformable using particle bombardment  
(Christou *et al.*, 1991). Furthermore, WO 93/21335 describes techniques for the  
transformation of rice via electroporation.

30 Patent Application EP 0 332 581 describes techniques for the generation,  
transformation and regeneration of Pooideae protoplasts. These techniques allow the  
transformation of *Dactylis* and wheat. Furthermore, wheat transformation has been  
described by Vasil *et al.* (1992) using particle bombardment into cells of type C long-term  
35 regenerable callus, and also by Vasil *et al.* (1993) and Weeks *et al.* (1993) using particle  
bombardment of immature embryos and immature embryo-derived callus. A preferred  
technique for wheat transformation, however, involves the transformation of wheat by  
particle bombardment of immature embryos and includes either a high sucrose or a high  
40 maltose step prior to gene delivery. Prior to bombardment, any number of embryos (0.75-1  
mm in length) are plated onto MS medium with 3% sucrose (Murashiga & Skoog, 1962) and  
3 mg/l 2,4-D for induction of somatic embryos, which is allowed to proceed in the dark. On  
45 the chosen day of bombardment, embryos are removed from the induction medium and  
placed onto the osmoticum (*i.e.* induction medium with sucrose or maltose added at the  
desired concentration, typically 15%). The embryos are allowed to plasmolyze for 2-3 h and  
are then bombarded. Twenty embryos per target plate is typical, although not critical. An  
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5 appropriate gene-carrying plasmid (such as pCIB3064 or pSG35) is precipitated onto  
micrometer size gold particles using standard procedures. Each plate of embryos is shot  
with the DuPont Biolistics® helium device using a burst pressure of ~1000 psi using a  
10 standard 80 mesh screen. After bombardment, the embryos are placed back into the dark  
to recover for about 24 h (still on osmoticum). After 24 hrs, the embryos are removed from  
the osmoticum and placed back onto induction medium where they stay for about a month  
before regeneration. Approximately one month later the embryo explants with developing  
15 embryogenic callus are transferred to regeneration medium (MS + 1 mg/liter NAA, 5 mg/liter  
GA), further containing the appropriate selection agent (10 mg/l basta in the case of  
pCIB3064 and 2 mg/l methotrexate in the case of pSOG35). After approximately one  
month, developed shoots are transferred to larger sterile containers known as "GA7s" which  
20 contain half-strength MS, 2% sucrose, and the same concentration of selection agent.

Transformation of monocotyledons using *Agrobacterium* has also been described.  
See, WO 94/00977 and U.S. Patent No. 5,591,616.

### 25 III. Breeding and Seed Production

#### Example 13: Breeding

30 The plants obtained via transformation with a gene of the present invention can be any  
of a wide variety of plant species, including those of monocots and dicots; however, the  
plants used in the method of the invention are preferably selected from the list of  
agronomically important target crops set forth *supra*. The expression of a gene of the  
35 present invention in combination with other characteristics important for production and  
quality can be incorporated into plant lines through breeding. Breeding approaches and  
techniques are known in the art. See, for example, Welsh J. R. (1981); Wood D. R. (Ed.)  
(1983); Mayo O. (1987); Singh, D.P. (1986); and Wricke and Weber (1986).

40 The genetic properties engineered into the transgenic seeds and plants described  
above are passed on by sexual reproduction or vegetative growth and can thus be  
maintained and propagated in progeny plants. Generally said maintenance and propagation  
45 make use of known agricultural methods developed to fit specific purposes such as tilling,  
sowing or harvesting. Specialized processes such as hydroponics or greenhouse  
technologies can also be applied. As the growing crop is vulnerable to attack and damages  
caused by insects or infections as well as to competition by weed plants, measures are  
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5 undertaken to control weeds, plant diseases, insects, nematodes, and other adverse  
conditions to improve yield. These include mechanical measures such a tillage of the soil or  
removal of weeds and infected plants, as well as the application of agrochemicals such as  
10 herbicides, fungicides, gametocides, nematocides, growth regulants, ripening agents and  
insecticides.

Use of the advantageous genetic properties of the transgenic plants and seeds  
according to the invention can further be made in plant breeding, which aims at the  
15 development of plants with improved properties such as tolerance of pests, herbicides, or  
stress, improved nutritional value, increased yield, or improved structure causing less loss  
from lodging or shattering. The various breeding steps are characterized by well-defined  
human intervention such as selecting the lines to be crossed, directing pollination of the  
20 parental lines, or selecting appropriate progeny plants. Depending on the desired  
properties, different breeding measures are taken. The relevant techniques are well known  
in the art and include but are not limited to hybridization, inbreeding, backcross breeding,  
multiline breeding, variety blend, interspecific hybridization, aneuploid techniques, etc.  
25 Hybridization techniques also include the sterilization of plants to yield male or female  
sterile plants by mechanical, chemical, or biochemical means. Cross pollination of a male  
sterile plant with pollen of a different line assures that the genome of the male sterile but  
female fertile plant will uniformly obtain properties of both parental lines. Thus, the  
30 transgenic seeds and plants according to the invention can be used for the breeding of  
improved plant lines, that for example, increase the effectiveness of conventional methods  
such as herbicide or pestidice treatment or allow one to dispense with said methods due to  
their modified genetic properties. Alternatively new crops with improved stress tolerance can  
35 be obtained, which, due to their optimized genetic "equipment", yield harvested product of  
better quality than products that were not able to tolerate comparable adverse  
developmental conditions.

#### 40 Example 14: Seed Production

In seeds production, germination quality and uniformity of seeds are essential  
45 product characteristics, whereas germination quality and uniformity of seeds harvested and  
sold by the farmer is not important. As it is difficult to keep a crop free from other crop and  
weed seeds, to control seedborne diseases, and to produce seed with good germination,  
fairly extensive and well-defined seed production practices have been developed by seed  
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5 producers, who are experienced in the art of growing, conditioning and marketing of pure  
seed. Thus, it is common practice for the farmer to buy certified seed meeting specific  
quality standards instead of using seed harvested from his own crop. Propagation material  
10 to be used as seeds is customarily treated with a protectant coating comprising herbicides,  
insecticides, fungicides, bactericides, nematocides, molluscicides, or mixtures thereof.  
Customarily used protectant coatings comprise compounds such as captan, carboxin,  
thiram (TMTD<sup>®</sup>), methalaxyl (Apron<sup>®</sup>), and pirimiphos-methyl (Actellic<sup>®</sup>). If desired, these  
15 compounds are formulated together with further carriers, surfactants or application-  
promoting adjuvants customarily employed in the art of formulation to provide protection  
against damage caused by bacterial, fungal or animal pests. The protectant coatings may  
be applied by impregnating propagation material with a liquid formulation or by coating with  
20 a combined wet or dry formulation. Other methods of application are also possible such as  
treatment directed at the buds or the fruit.

It is a further aspect of the present invention to provide new agricultural methods,  
such as the methods exemplified above, which are characterized by the use of transgenic  
25 plants, transgenic plant material, or transgenic seed according to the present invention.

The seeds may be provided in a bag, container or vessel comprised of a suitable  
packaging material, the bag or container capable of being closed to contain seeds. The  
bag, container or vessel may be designed for either short term or long term storage, or both,  
30 of the seed. Examples of a suitable packaging material include paper, such as kraft paper,  
rigid or pliable plastic or other polymeric material, glass or metal. Desirably the bag,  
container, or vessel is comprised of a plurality of layers of packaging materials, of the same  
or differing type. In one embodiment the bag, container or vessel is provided so as to  
35 exclude or limit water and moisture from contacting the seed. In one example, the bag,  
container or vessel is sealed, for example heat sealed, to prevent water or moisture from  
entering. In another embodiment water absorbent materials are placed between or  
adjacent to packaging material layers. In yet another embodiment the bag, container or  
40 vessel, or packaging material of which it is comprised is treated to limit, suppress or  
prevent disease, contamination or other adverse affects of storage or transport of the seed.  
An example of such treatment is sterilization, for example by chemical means or by  
45 exposure to radiation. Comprised by the present invention is a commercial bag comprising  
seed of a transgenic plant comprising a gene of the present invention that is expressed in  
said transformed plant at higher levels than in a wild type plant, together with a suitable



5 carrier, together with label instructions for the use thereof for conferring broad spectrum  
disease resistance to plants.

#### 10 IV. Disease Resistance Evaluation

Disease resistance evaluation is performed by methods known in the art. See, Uknes  
15 *et al.* (1993); Görlach *et al.* (1996); Alexander *et al.* (1993). For example, several  
representative disease resistance assays are described below.

##### Example 15: *Phytophthora parasitica* (Black Shank) Resistance Assay

20 Assays for resistance to *Phytophthora parasitica*, the causative organism of black  
shank, are performed on six-week-old plants grown as described in Alexander *et al.* (1993).  
Plants are watered, allowed to drain well, and then inoculated by applying 10 ml of a  
sporangium suspension (300 sporangia/ml) to the soil. Inoculated plants are kept in a  
25 greenhouse maintained at 23-25°C day temperature, and 20-22°C night temperature. The  
wilt index used for the assay is as follows: 0=no symptoms; 1=no symptoms; 1=some sign  
of wilting, with reduced turgidity; 2=clear wilting symptoms, but no rotting or stunting;  
3=clear wilting symptoms with stunting, but no apparent stem rot; 4=severe wilting, with  
30 visible stem rot and some damage to root system; 5=as for 4, but plants near death or  
dead, and with severe reduction of root system. All assays are scored blind on plants  
arrayed in a random design.

##### 35 Example 16: *Pseudomonas syringae* Resistance Assay

*Pseudomonas syringae* pv. *tabaci* strain #551 is injected into the two lower leaves of  
40 several 6-7-week-old plants at a concentration of  $10^6$  or  $3 \times 10^6$  per ml in H<sub>2</sub>O. Six individual  
plants are evaluated at each time point. *Pseudomonas tabaci* infected plants are rated on a  
5 point disease severity scale, 5=100% dead tissue, 0=no symptoms. A T-test (LSD) is  
conducted on the evaluations for each day and the groupings are indicated after the Mean  
45 disease rating value. Values followed by the same letter on that day of evaluation are not  
statistically significantly different.

Example 17: *Cercospora nicotianae* Resistance Assay

A spore suspension of *Cercospora nicotianae* (ATCC #18366) (100,000-150,000 spores per ml) is sprayed to imminent run-off onto the surface of the leaves. The plants are maintained in 100% humidity for five days. Thereafter the plants are misted with water 5-10 times per day. Six individual plants are evaluated at each time point. *Cercospora nicotianae* is rated on a % leaf area showing disease symptoms basis. A T-test (LSD) is conducted on the evaluations for each day and the groupings are indicated after the Mean disease rating value. Values followed by the same letter on that day of evaluation are not statistically significantly different.

Example 18: *Peronospora parasitica* Resistance Assay

Assays for resistance to *Peronospora parasitica* are performed on plants as described in Uknes *et al.*, (1993). Plants are inoculated with a compatible isolate of *P. parasitica* by spraying with a conidial suspension (approximately  $5 \times 10^4$  spores per milliliter). Inoculated plants are incubated under humid conditions at 17° C in a growth chamber with a 14-hr day/10-hr night cycle. Plants are examined at 3-14 days, preferably 7-12 days, after inoculation for the presence of conidiophores. In addition, several plants from each treatment are randomly selected and stained with lactophenol-trypan blue (Keogh *et al.*, 1980) for microscopic examination.

The above disclosed embodiments are illustrative. This disclosure of the invention will place one skilled in the art in possession of many variations of the invention. All such obvious and foreseeable variations are intended to be encompassed by the claims.

## BRIEF DESCRIPTION OF THE SEQUENCES IN THE SEQUENCE LISTING

SEQ ID NO:1 - Full length cDNA sequence of a *NIM1* homologue from *Nicotiana tabacum*.

SEQ ID NO:2 - Protein sequence of the *Nicotiana tabacum* NIM1 homologue encoded by SEQ ID NO:1.

SEQ ID NO:3 - Full length cDNA sequence of a *NIM1* homologue from *Lycopersicon esculentum*.

- 5 SEQ ID NO:4 - Protein sequence of the *Lycopersicon esculentum* NIM1 homologue encoded by SEQ ID NO:3.
- SEQ ID NO:5 - Partial cDNA sequence of a *NIM1* homologue from *Brassica napus*.
- 10 SEQ ID NO:6 - Partial protein sequence of the *Brassica napus* NIM1 homologue encoded by SEQ ID NO:5.
- SEQ ID NO:7 - Full length cDNA sequence of a *NIM1* homologue (*AtNMLc5*) from *Arabidopsis thaliana*.
- 15 SEQ ID NO:8 - Full length protein sequence of the *Arabidopsis thaliana* NIM1 homologue *AtNMLc5* encoded by SEQ ID NO:7.
- SEQ ID NOs:9-14 - Oligonucleotide primers used in Examples 1-4.
- SEQ ID NO:15 - Genomic DNA sequence of a *NIM1* homologue (*AtNMLc2*) from *Arabidopsis thaliana*.
- 20 SEQ ID NO:16 - Protein sequence of the *Arabidopsis thaliana* NIM1 homologue *AtNMLc2* encoded by SEQ ID NO:15.
- SEQ ID NO:17 - Genomic DNA sequence of a *NIM1* homologue (*AtNMLc4-1*) from *Arabidopsis thaliana*.
- 25 SEQ ID NO:18 - Protein sequence of the *Arabidopsis thaliana* NIM1 homologue *AtNMLc4-1* encoded by SEQ ID NO:17.
- SEQ ID NO:19 - Genomic DNA sequence of a *NIM1* homologue (*AtNMLc4-2*) from *Arabidopsis thaliana*.
- 30 SEQ ID NO:20 - Protein sequence of the *Arabidopsis thaliana* NIM1 homologue *AtNMLc4-2* encoded by SEQ ID NO:19.
- 35 SEQ ID NO:21 - PCR primer NIM 1A.
- SEQ ID NO:22 - PCR primer NIM 1B.
- SEQ ID NO:23 - PCR primer NIM 1C.
- SEQ ID NO:24 - PCR primer NIM 1D.
- 40 SEQ ID NO:25 - PCR primer NIM 2A.
- SEQ ID NO:26 - PCR primer NIM 2B.
- SEQ ID NO:27 - PCR primer NIM 2C.
- SEQ ID NO:28 - PCR primer NIM 2D.
- 45 SEQ ID NO:29 - 659 bp *NIM*-like DNA fragment amplified from *Nicotiana tabacum* (Tobacco A), which is a consensus of 36 sequences and has 67% sequence identity to the *Arabidopsis thaliana* *NIM1* gene sequence.
- 50

- 5 SEQ ID NO:30 - Protein sequence encoded by SEQ ID NO:29.
- SEQ ID NO:31 - 498 bp *NIM*-like DNA fragment amplified from *Nicotiana tabacum* (Tobacco B), which is a consensus of 2 sequences and has 62% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 10 SEQ ID NO:32 - Protein sequence encoded by SEQ ID NO:31.
- SEQ ID NO:33 - 498 bp *NIM*-like DNA fragment amplified from *Nicotiana tabacum* (Tobacco C), which is a consensus of 3 sequences and has 63% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 15 SEQ ID NO:34 - Protein sequence encoded by SEQ ID NO:33.
- SEQ ID NO:35 - 399 bp *NIM*-like DNA fragment amplified from *Nicotiana tabacum* (Tobacco D), which has 59% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 20 SEQ ID NO:36 - Protein sequence encoded by SEQ ID NO:35.
- SEQ ID NO:37 - 498 bp *NIM*-like DNA fragment amplified from *Lycopersicon esculentum* (Tomato A), which is a consensus of 8 sequences and has 67% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 25 SEQ ID NO:38 - Protein sequence encoded by SEQ ID NO:37.
- SEQ ID NO:39 - 498 bp *NIM*-like DNA fragment amplified from *Beta vulgaris* (Sugarbeet), which is a consensus of 24 sequences and has 66% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 30 SEQ ID NO:40 - Protein sequence encoded by SEQ ID NO:39.
- SEQ ID NO:41 - 498 bp *NIM*-like DNA fragment amplified from *Helianthus annuus* (Sunflower A), which is a consensus of 9 sequences and has 61% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 35 SEQ ID NO:42 - Protein sequence encoded by SEQ ID NO:41.
- SEQ ID NO:43 - 498 bp *NIM*-like DNA fragment amplified from *Helianthus annuus* (Sunflower B), which is a consensus of 10 sequences and has 59% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 40 SEQ ID NO:44 - Protein sequence encoded by SEQ ID NO:43.
- SEQ ID NO:45 - 653 bp *NIM*-like DNA fragment amplified from *Solanum tuberosum* (Potato A), which is a consensus of 15 sequences and has 68% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.
- 45 SEQ ID NO:46 - Protein sequence encoded by SEQ ID NO:45.
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5 SEQ ID NO:47 - 498 bp *NIM*-like DNA fragment amplified from *Solanum tuberosum* (Potato B), which is a consensus of 3 sequences and has 61% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

SEQ ID NO:48 - Protein sequence encoded by SEQ ID NO:47.

10 SEQ ID NO:49 - 477 bp *NIM*-like DNA fragment amplified from *Solanum tuberosum* (Potato C), which is a consensus of 2 sequences and has 62% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

SEQ ID NO:50 - Protein sequence encoded by SEQ ID NO:49.

15 SEQ ID NO:51 - 501 bp *NIM*-like DNA fragment amplified from *Brassica napus* (Canola A), which is a consensus of 5 sequences and has 59% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

SEQ ID NO:52 - Protein sequence encoded by SEQ ID NO:51.

20 SEQ ID NO:53 - 501 bp *NIM*-like DNA fragment amplified from *Brassica napus* (Canola B), which is a consensus of 5 sequences and has 58% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

25 SEQ ID NO:54 - Protein sequence encoded by SEQ ID NO:53.

SEQ ID NO:55 - 498 bp *NIM*-like DNA fragment amplified from *Brassica napus* (Canola C), which has 56% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

30 SEQ ID NO:56 - Protein sequence encoded by SEQ ID NO:55.

SEQ ID NO:57 - 498 bp *NIM*-like DNA fragment amplified from *Brassica napus* (Canola D), which has 73% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

35 SEQ ID NO:58 - Protein sequence encoded by SEQ ID NO:57.

SEQ ID NO:59 - PCR primer NIM 3A.

SEQ ID NO:60 - PCR primer NIM 3B.

40 SEQ ID NO:61 - 148 bp *NIM*-like DNA fragment amplified from *Lycopersicon esculentum* (Tomato B), which is a consensus of 3 sequences and has 72% sequence identity to the *Arabidopsis thaliana NIM1* gene sequence.

SEQ ID NO:62 - Protein sequence encoded by SEQ ID NO:61.

45 SEQ ID NO:63 - Full length cDNA sequence of a *NIM1* homologue from *Beta vulgaris* (Sugarbeet), which corresponds to the PCR fragment of SEQ ID NO:39.

SEQ ID NO:64 - Protein sequence of the sugarbeet *NIM1* homologue encoded by SEQ ID NO:62.

5 SEQ ID NO:65 - Full length cDNA sequence of a *NIM1* homologue from *Helianthus annuus*  
(Sunflower B), which corresponds to the PCR fragment of SEQ ID NO:43.  
SEQ ID NO:66 - Protein sequence of the *Helianthus annuus* NIM1 homologue encoded by  
SEQ ID NO:65.  
10 SEQ ID NO:67 - cDNA sequence corresponding to the *Arabidopsis thaliana* NIM-like  
genomic sequence *AtNMLc2* (SEQ ID NO:15).  
SEQ ID NO:68 - Protein sequence encoded by SEQ ID NO:67.  
SEQ ID NO:69 - cDNA sequence corresponding to the *Arabidopsis thaliana* NIM-like  
15 genomic sequence *AtNMLc4-1* (SEQ ID NO:17).  
SEQ ID NO:70 - Protein sequence encoded by SEQ ID NO:69.  
SEQ ID NO:71 - cDNA sequence corresponding to the *Arabidopsis thaliana* NIM-like  
genomic sequence *AtNMLc4-2* (SEQ ID NO:19).  
20 SEQ ID NO:72 - Protein sequence encoded by SEQ ID NO:71.  
SEQ ID NO:73 - Full length cDNA sequence of a *NIM1* homologue from *Nicotiana tabacum*  
(Tobacco B), which corresponds to the PCR fragment of SEQ ID NO:31.  
25 SEQ ID NO:74 - Protein sequence of the *Nicotiana tabacum* NIM1 homologue encoded by  
SEQ ID NO:73.

## DEPOSITS

30 The following material has been deposited with the Agricultural Research Service,  
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35 Microorganisms for the Purposes of Patent Procedure. All restrictions on the availability of  
the deposited material will be irrevocably removed upon the granting of a patent.

<u>Clone</u>	<u>Accession Number</u>	<u>Date of Deposit</u>
pNOV1203	NRRL B-30049	August 17, 1998
pNOV1204	NRRL B-30050	August 17, 1998
pNOV1206	NRRL B-30051	August 17, 1998
45 AtNMLc5	NRRL B-30139	May 25, 1999

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The references cited herein are indicative of the current state of the art. Each of the following is incorporated by reference into the instant disclosure.

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D. DESIGNATED STATES FOR WHICH INDICATIONS ARE MADE (if the indications are not for all designated States)	
E. SEPARATE FURNISHING OF INDICATIONS (leave blank if not applicable)	
The indications listed below will be submitted to the International Bureau later (specify the general nature of the indications e.g., "Accession Number of Deposit")	
<b>For receiving Office use only</b>	<b>For International Bureau use only</b>
<input checked="" type="checkbox"/> This sheet was received with the international application <b>07 MAR 2000</b>	<input type="checkbox"/> This sheet was received by the International Bureau on:
Authorized officer <b>E. Speiser ES</b>	Authorized officer



## Claims

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## What Is Claimed Is:

## 1. An isolated nucleic acid molecule comprising:

(a) a nucleotide sequence that encodes SEQ ID NO:2, 4, 6, 8, 16, 18, 20, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 62, 64, 66, 68, 70, 72, or 74;

(b) SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73;

(c) a nucleotide sequence that comprises an at least 20 consecutive base pair portion identical in sequence to an at least 20 consecutive base pair portion of SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73;

(d) a nucleotide sequence that can be amplified from a *Lycopersicon esculentum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, SEQ ID NO:26 and 28, or SEQ ID NO:59 and 60;

(e) a nucleotide sequence that can be amplified from a *Beta vulgaris* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:22 and 24 or SEQ ID NO:26 and 28;

(f) a nucleotide sequence that can be amplified from a *Helianthus annuus* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:26 and 28;

(g) a nucleotide sequence that can be amplified from a *Solanum tuberosum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:21 and 24, SEQ ID NO:21 and 23, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, or SEQ ID NO:26 and 28;

(h) a nucleotide sequence that can be amplified from a *Brassica napus* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10 or SEQ ID NO:26 and 28;

(i) a nucleotide sequence that can be amplified from an *Arabidopsis thaliana* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:13 and 14, SEQ ID NO:21 and 24, or SEQ ID NO:22 and 24;

(j) a nucleotide sequence that can be amplified from an *Nicotiana tabacum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID

5 NO:9 and 10, SEQ ID NO:11 and 12, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, or SEQ ID NO:26 and 28; or

10 (k) a nucleotide sequence that can be amplified from an plant DNA library using the polymerase chain reaction with a pair of primers comprising the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73.

15 2. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that encodes SEQ ID NO:2, 4, 6, 8, 16, 18, 20, 30, 32, 34, 36, 38, 40, 42, 44, 46, 48, 50, 52, 54, 56, 58, 62, 64, 66, 68, 70, 72, or 74.

20 3. An isolated nucleic acid molecule according to claim 1, comprising SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73.

25 4. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that comprises an at least 20 consecutive base pair portion identical in sequence to an at least 20 consecutive base pair portion of SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73.

30 5. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from a *Lycopersicon esculentum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, SEQ ID NO:26 and 28, or SEQ ID NO:59 and 60.

35 40 6. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from a *Beta vulgaris* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:22 and 24 or SEQ ID NO:26 and 28.

5 7. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from a *Helianthus annuus* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:26 and 28.

10 8. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from a *Solanum tuberosum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:21 and 24, SEQ ID NO:21 and 23, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, or SEQ ID NO:26 and 28.

15 9. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from a *Brassica napus* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10 or SEQ ID NO:26 and 28.

20 10. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from an *Arabidopsis thaliana* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:13 and 14, SEQ ID NO:21 and 24, or SEQ ID NO:22 and 24.

25 11. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from an *Nicotiana tabacum* DNA library using the polymerase chain reaction with the pair of primers set forth as SEQ ID NO:9 and 10, SEQ ID NO:11 and 12, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ ID NO:25 and 28, or SEQ ID NO:26 and 28.

30 12. An isolated nucleic acid molecule according to claim 1, comprising a nucleotide sequence that can be amplified from a plant DNA library using the polymerase chain reaction with a pair of primers corresponding to the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 45 71, or 73.

50 13. A chimeric gene comprising a promoter active in plants operatively linked to a nucleic acid molecule according to any one of the preceding claims.

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14. A recombinant vector comprising the chimeric gene of claim 13.

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15. A host cell comprising the chimeric gene of claim 13.

16. A plant comprising the chimeric gene of claim 13.

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17. The plant of claim 16, which is selected from the following: rice, wheat, barley, rye, corn, potato, canola, sunflower, carrot, sweet potato, sugarbeet, bean, pea, chicory, lettuce, cabbage, cauliflower, broccoli, turnip, radish, spinach, asparagus, onion, garlic, eggplant, pepper, celery, squash, pumpkin, cucumber, apple, pear, quince, melon, plum, cherry, peach, nectarine, apricot, strawberry, grape, raspberry, blackberry, pineapple, avocado, papaya, mango, banana, soybean, tobacco, tomato, sorghum and sugarcane.

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18. Seed from the plant of claim 16.

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19. A method of increasing SAR gene expression in a plant, comprising expressing the chimeric gene of claim 13 in said plant.

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20. A method of enhancing disease resistance in a plant, comprising expressing the chimeric gene of claim 13 in said plant.

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21. A PCR primer selected from the group consisting of SEQ ID NO:9-14, 21-28, 59, and 60.

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22. A method for isolating a *NIM1* homologue involved in the signal transduction cascade leading to systemic acquired resistance in plants comprising amplifying a DNA molecule from a plant DNA library using the polymerase chain reaction with a pair of primers corresponding to the first 20 nucleotides and the reverse complement of the last 20 nucleotides of the coding sequence (CDS) of SEQ ID NO:1, 3, 5, 7, 15, 17, 19, 29, 31, 33, 35, 37, 39, 41, 43, 45, 47, 49, 51, 53, 55, 57, 61, 63, 65, 67, 69, 71, or 73 or with the pair of primers set forth as SEQ ID NO:9 and 10, SEQ ID NO:11 and 12, SEQ ID NO:13 and 14, SEQ ID NO:21 and 24, SEQ ID NO:22 and 24, SEQ ID NO:21 and 23, SEQ ID NO:25 and 28, SEQ ID NO:26 and 28, or SEQ ID NO:59 and 60.

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23. The method of claim 22, wherein said plant DNA library is a *Nicotiana tabacum* (tobacco), *Lycopersicon esculentum* (tomato), *Brassica napus* (oilseed rape), *Arabidopsis thaliana*, *Beta vulgaris* (sugarbeet), *Helianthus annuus* (sunflower), or *Solanum tuberosum* (potato) DNA library.

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## SEQUENCE LISTING

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&lt;120&gt; NOVEL PLANT GENES AND USES THEREOF

&lt;130&gt; S-30857A/RTP2095

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&lt;141&gt;

&lt;160&gt; 74

&lt;170&gt; PatentIn Ver. 2.1

&lt;210&gt; 1

&lt;211&gt; 1767

&lt;212&gt; DNA

&lt;213&gt; Nicotiana tabacum

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (1)..(1764)

&lt;223&gt; Full length tobacco cDNA sequence

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Pro Glu Thr Ser Pro Ala Glu Ile Thr Ser Leu Lys Arg Leu Ser Glu	
35 40 45	
aca ctg gaa tct atc ttc gat gcg tct ttg ccg gag ttt gac tac ttc	192
Thr Leu Glu Ser Ile Phe Asp Ala Ser Leu Pro Glu Phe Asp Tyr Phe	
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Ala Asp Ala Lys Leu Val Val Ser Gly Pro Cys Lys Glu Ile Pro Val	
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Ser Ser Ile Cys Cys Met Asn Glu Ser Glu Thr Ser Leu Ala Asp Val	
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Asn Ser Leu Lys Arg Leu Ser Glu Thr Leu Glu Ser Ile Phe Asp Ala	
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Ala	Leu	Gln	Ile	Ala	Lys	Arg	Leu	Thr	Arg	Leu	Val	Asp	Phe	Thr	Lys	
		355					360					365				
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Ser	Thr	Glu	Glu	Gly	Lys	Ser	Ala	Pro	Lys	Asp	Arg	Leu	Cys	Ile	Glu	
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Ile	Leu	Glu	Gln	Ala	Glu	Arg	Arg	Asp	Pro	Leu	Leu	Gly	Glu	Ala	Ser	
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Leu	Ser	Leu	Ala	Met	Ala	Gly	Asp	Asp	Leu	Arg	Met	Lys	Leu	Leu	Tyr	
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ctt	gaa	aat	aga	gtt	ggt	ctg	gct	aaa	ctc	ctt	ttt	ccc	atg	gaa	gca	1296
Leu	Glu	Asn	Arg	Val	Gly	Leu	Ala	Lys	Leu	Leu	Phe	Pro	Met	Glu	Ala	
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Lys	Val	Ala	Met	Asp	Ile	Ala	Gln	Val	Asp	Gly	Thr	Ser	Glu	Leu	Pro	
		435					440					445				
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Leu	Ala	Ser	Met	Arg	Lys	Lys	Ile	Ala	Asp	Ala	Gln	Arg	Thr	Thr	Val	
	450					455					460					
gat	ttg	aac	gag	gct	cct	ttc	aag	atg	aaa	gag	gag	cac	ttg	aat	cgg	1440
Asp	Leu	Asn	Glu	Ala	Pro	Phe	Lys	Met	Lys	Glu	Glu	His	Leu	Asn	Arg	
	465				470					475				480		
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Leu	Arg	Ala	Leu	Ser	Arg	Thr	Val	Glu	Leu	Gly	Lys	Arg	Phe	Phe	Pro	

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Arg Cys Ser Glu Val Leu Asn Lys Ile Met Asp Ala Asp Asp Leu Ser				
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Glu Ile Ala Tyr Met Gly Asn Asp Thr Val Glu Glu Arg Gln Leu Lys				
	515	520	525	
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Lys Gln Arg Tyr Met Glu Leu Gln Glu Ile Leu Ser Lys Ala Phe Thr				
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Glu Asp Lys Glu Glu Phe Ala Lys Thr Asn Met Ser Ser Ser Cys Ser				
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tct aca tct aag gga gta gat aag ccc aat aat ctc cca ttt agg aaa				1728
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Gly Gly Lys Glu Ile Pro Val His Arg Cys Ile Leu Ser Ala Arg Ser				
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Pro Phe Phe Lys Asn Val Phe Cys Gly Lys Asp Ser Ser Thr Lys Leu				
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Lys Asp Val Cys Val Cys Val Asp Asn Glu Cys Leu His Val Ala Cys				
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Arg Pro Ala Val Ala Phe Met Val Gln Val Leu Tyr Ala Ser Phe Thr				
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 Ala Asn Ile Cys Gly Lys Ala Cys Glu Arg Leu Leu Ser Arg Cys Ile  
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 Asp Ile Ile Val Lys Ser Asn Val Asp Ile Ile Thr Leu Asp Lys Ser  
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 Leu Pro His Asp Ile Val Lys Gln Ile Thr Asp Ser Arg Ala Glu Leu  
 225 230 235 240  
 Gly Leu Gln Gly Pro Glu Ser Asn Gly Phe Pro Asp Lys His Val Lys  
 245 250 255  
 Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Leu Arg Met  
 260 265 270  
 Leu Leu Lys Glu Gly His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His  
 275 280 285  
 Tyr Ala Val Ala Tyr Cys Asp Ala Lys Thr Thr Ala Glu Leu Leu Asp  
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 Ser Thr Glu Glu Gly Lys Ser Ala Pro Lys Asp Arg Leu Cys Ile Glu  
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 385 390 395 400  
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 Leu Glu Asn Arg Val Gly Leu Ala Lys Leu Leu Phe Pro Met Glu Ala  
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 Lys Val Ala Met Asp Ile Ala Gln Val Asp Gly Thr Ser Glu Leu Pro  
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 Leu Ala Ser Met Arg Lys Lys Ile Ala Asp Ala Gln Arg Thr Thr Val  
 450 455 460  
 Asp Leu Asn Glu Ala Pro Phe Lys Met Lys Glu Glu His Leu Asn Arg  
 465 470 475 480  
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	485		490		495
Arg Cys Ser	Glu Val Leu Asn Lys	Ile Met Asp Ala Asp	Asp Leu Ser		
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Glu Ile Ala	Tyr Met Gly Asn Asp Thr Val Glu Glu Arg Gln Leu Lys				
	515	520	525		
Lys Gln Arg Tyr Met Glu Leu Gln Glu Ile Leu Ser Lys Ala Phe Thr					
	530	535	540		
Glu Asp Lys Glu Glu Phe Ala Lys Thr Asn Met Ser Ser Ser Cys Ser					
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gtc ttc ccg acg gag ctt ytc acc aga ccc gag gta tcc gcg ttt caa 144  
 Val Xaa Pro Thr Glu Leu Xaa Thr Arg Pro Glu Val Ser Ala Phe Gln  
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ctc ctc tcc aac agc ctc gag tcc gtc ttc gac tcg ccg gaa gcg ttc 192  
 Leu Leu Ser Asn Ser Leu Glu Ser Val Phe Asp Ser Pro Glu Ala Phe  
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tac agc gac gcc aag ctt gtt ctc tcc gac gac aag gaa gta tcc ttc 240  
 Tyr Ser Asp Ala Lys Leu Val Leu Ser Asp Asp Lys Glu Val Ser Phe  
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cac cgt tgc att ctc tcg gcg aga agc ctc ttc ttc aag gcc gct ttg 288  
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 Xaa Ala Ala Glu Lys Val Gln Lys Ser Thr Pro Val Lys Leu Glu Leu  
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aag aca ctc gcg gcg gaa tac gac gtc ggg ttc gat tct gtg gtg gct 384  
 Lys Thr Leu Ala Ala Glu Tyr Asp Val Gly Phe Asp Ser Val Val Ala  
 115 120 125



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Val Ser Glu Cys Ala Asp Xaa Ser Cys Cys His Val Ala Cys Arg Pro	
145 150 155 160	
gct gtg gat ttc atg gtg gag gtt ctc tac ttg gct ttc gtc ttc cag	528
Ala Val Asp Phe Met Val Glu Val Leu Tyr Leu Ala Phe Val Phe Gln	
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att cag gaa ctg gtt acc atg tat cag agg cat tta ctg gat gtt gta	576
Ile Gln Glu Leu Val Thr Met Tyr Gln Arg His Leu Leu Asp Val Val	
180 185 190	
gac aaa gtt awc ata gaa gac act ttg gtc gtc ctc aag ctt gct aac	624
Asp Lys Val Xaa Ile Glu Asp Thr Leu Val Val Leu Lys Leu Ala Asn	
195 200 205	
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Ile Cys Gly Lys Ala Cys Lys Lys Leu Phe Asp Lys Cys Arg Glu Ile	
210 215 220	
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Ile Val Lys Ser Asn Val Asp Val Val Thr Leu Lys Lys Ser Leu Pro	
225 230 235 240	
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Glu Xaa Ile Ala Lys Gln Val Ile Asp Ile Arg Lys Glu Leu Gly Leu	
245 250 255	
gag gta gct gaa cca gag aaa cat gtc tcc aac ata cac aag gcg ctt	816
Glu Val Ala Glu Pro Glu Lys His Val Ser Asn Ile His Lys Ala Leu	
260 265 270	
gag tca gac gat ctt gac ctt gtc gtt atg ctt ttg aaa gag ggc cac	864
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Thr Asn Leu Asp Glu Ala Tyr Ala Leu His Phe Ala Val Ala Tyr Cys	
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gat gag aag aca gcg agg aat ctc ctg gaa ctg ggg ttt gcg gat gtc	960
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Asn Arg Arg Asn Pro Arg Gly Tyr Thr Val Ile His Val Ala Ala Met	
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Arg Lys Glu Pro Thr Leu Ile Ala Leu Leu Thr Lys Gly Ala Asn	
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Ala Leu Glu Met Ser Leu Asp Gly Arg Thr Ala Leu Leu Ile Ala Lys	
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Gln Val Thr Lys Ala Ala Glu Cys Cys Ile Leu Glu Lys Gly Lys Leu
370 375 380

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Ala Ala Lys Gly Gly Val Cys Val Glu Ile Leu Lys Gln Pro Asp Asn
385 390 395 400

aca cga gaa cca ttt cct gaa gat gtt tct ccc tcc ctt gca gtg gct 1248
Thr Arg Glu Pro Phe Pro Glu Asp Val Ser Pro Ser Leu Ala Val Ala
405 410 415

gct gat caa ttc aag ata agg ttg att gat ctt gaa aac aga gtt caa 1296
Ala Asp Gln Phe Lys Ile Arg Leu Ile Asp Leu Glu Asn Arg Val Gln
420 425 430

atg gct cga tgt ctc tat cca atg gaa gca caa gtt gca atg gat ttc 1344
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gcc cga atg aag gga aca cgc gag ttt gtc gtg acg aca gca act gac 1392
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cta cac atg gaa cct ttc aag ttc gta gaa atg cat cag agt aga cta 1440
Leu His Met Glu Pro Phe Lys Phe Val Glu Met His Gln Ser Arg Leu
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Thr Ala Leu Ser Lys Thr Val Glu Phe Gly Lys Arg Phe Phe Pro Arg
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Cys Ser Lys Val Leu Asp Asp Ile Val Asp Ser Glu Asp Leu Thr Ile
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ctg gct ctc gta gaa gaa gac act cct gag caa cga caa caa aag agg 1584
Leu Ala Leu Val Glu Glu Asp Thr Pro Glu Gln Arg Gln Gln Lys Arg
515 520 525

cag agg ttc atg gaa ata cag gag att gtt caa atg gcg ttt agt aaa 1632
Gln Arg Phe Met Glu Ile Gln Glu Ile Val Gln Met Ala Phe Ser Lys
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Asp Lys Glu Asp Leu Gly Lys Ser Ser Leu Ser Ala Ser Ser Ser Ser
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Val Xaa Pro Thr Glu Leu Xaa Thr Arg Pro Glu Val Ser Ala Phe Gln
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Leu Leu Ser Asn Ser Leu Glu Ser Val Phe Asp Ser Pro Glu Ala Phe
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Tyr Ser Asp Ala Lys Leu Val Leu Ser Asp Asp Lys Glu Val Ser Phe
  65           70           75           80

His Arg Cys Ile Leu Ser Ala Arg Ser Leu Phe Phe Lys Ala Ala Leu
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Xaa Ala Ala Glu Lys Val Gln Lys Ser Thr Pro Val Lys Leu Glu Leu
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Lys Thr Leu Ala Ala Glu Tyr Asp Val Gly Phe Asp Ser Val Val Ala
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Val Leu Ala Tyr Val Tyr Ser Gly Arg Val Arg Pro Pro Pro Lys Gly
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Val Ser Glu Cys Ala Asp Xaa Ser Cys Cys His Val Ala Cys Arg Pro
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Ala Val Asp Phe Met Val Glu Val Leu Tyr Leu Ala Phe Val Phe Gln
      165          170          175

Ile Gln Glu Leu Val Thr Met Tyr Gln Arg His Leu Leu Asp Val Val
      180          185          190

Asp Lys Val Xaa Ile Glu Asp Thr Leu Val Val Leu Lys Leu Ala Asn
      195          200          205

Ile Cys Gly Lys Ala Cys Lys Lys Leu Phe Asp Lys Cys Arg Glu Ile
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Ile Val Lys Ser Asn Val Asp Val Val Thr Leu Lys Lys Ser Leu Pro
      225          230          235          240

Glu Xaa Ile Ala Lys Gln Val Ile Asp Ile Arg Lys Glu Leu Gly Leu
      245          250          255

Glu Val Ala Glu Pro Glu Lys His Val Ser Asn Ile His Lys Ala Leu
      260          265          270

Glu Ser Asp Asp Leu Asp Leu Val Val Met Leu Leu Lys Glu Gly His
      275          280          285

Thr Asn Leu Asp Glu Ala Tyr Ala Leu His Phe Ala Val Ala Tyr Cys
      290          295          300

Asp Glu Lys Thr Ala Arg Asn Leu Leu Glu Leu Gly Phe Ala Asp Val
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 355 360 365  
 Gln Val Thr Lys Ala Ala Glu Cys Cys Ile Leu Glu Lys Gly Lys Leu  
 370 375 380  
 Ala Ala Lys Gly Gly Val Cys Val Glu Ile Leu Lys Gln Pro Asp Asn  
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 Thr Arg Glu Pro Phe Pro Glu Asp Val Ser Pro Ser Leu Ala Val Ala  
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 Cys Ser Lys Val Leu Asp Asp Ile Val Asp Ser Glu Asp Leu Thr Ile  
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Arg Ser Lys Phe Phe Gln Asp Leu Phe Lys Lys Glu Lys Lys Ile Ser	
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Lys Thr Glu Lys Pro Lys Tyr Gln Leu Arg Glu Met Leu Pro Tyr Gly	
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Ala Val Ala His Glu Ala Phe Leu Tyr Phe Leu Ser Tyr Ile Tyr Thr	
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Gly Arg Leu Lys Pro Phe Pro Leu Glu Val Ser Thr Cys Val Asp Pro	
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Val Cys Ser His Asp Cys Cys Arg Pro Ala Ile Asp Phe Val Val Gln	
145 150 155 160	
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gtt ctt ccc att ctt atg gtt gct ttc aat tgt aag ttg act cag ctt	624
Val Leu Pro Ile Leu Met Val Ala Phe Asn Cys Lys Leu Thr Gln Leu	
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Cys Ile Glu Lys Glu Val Pro Pro Glu Val Ala Glu Lys Ile Lys Gln	

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	260	265	270	
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	340	345	350	
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	370	375	380	
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	420	425	430	
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	465	470	475	480

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 tac atg gct gag tat ata gac gac gac atc ctc gac gat ttc cat ttt 1584  
 Tyr Met Ala Glu Tyr Ile Asp Asp Ile Leu Asp Asp Phe His Phe  
 515 520 525  
 gag aag gga tct aca cat gaa aga aga ttg aaa aga atg aga tat aga 1632  
 Glu Lys Gly Ser Thr His Glu Arg Arg Leu Lys Arg Met Arg Tyr Arg  
 530 535 540  
 gag ctt aag gat gat gtc caa aag gca tat agc aaa gac aaa gag tct 1680  
 Glu Leu Lys Asp Asp Val Gln Lys Ala Tyr Ser Lys Asp Lys Glu Ser  
 545 550 555 560  
 aag att gcg cgg tct tgt ctt tct gct tca tct tct cct tct tct tct 1728  
 Lys Ile Ala Arg Ser Cys Leu Ser Ala Ser Ser Ser Pro Ser Ser Ser  
 565 570 575  
 tcc ata aga gat gat ctg cac aac aca aca tga 1761  
 Ser Ile Arg Asp Asp Leu His Asn Thr Thr  
 580 585

<210> 8  
 <211> 586  
 <212> PRT  
 <213> Arabidopsis thaliana

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 Met Ala Thr Leu Thr Glu Pro Ser Ser Ser Leu Ser Phe Thr Ser Ser  
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 His Phe Ser Tyr Gly Ser Ile Gly Ser Asn His Phe Ser Ser Ser Ser  
 20 25 30  
 Ala Ser Asn Pro Glu Val Val Ser Leu Thr Lys Leu Ser Ser Asn Leu  
 35 40 45  
 Glu Gln Leu Leu Ser Asn Ser Asp Cys Asp Tyr Ser Asp Ala Glu Ile  
 50 55 60  
 Ile Val Asp Gly Val Pro Val Gly Val His Arg Cys Ile Leu Ala Ala  
 65 70 75 80  
 Arg Ser Lys Phe Phe Gln Asp Leu Phe Lys Lys Glu Lys Lys Ile Ser  
 85 90 95  
 Lys Thr Glu Lys Pro Lys Tyr Gln Leu Arg Glu Met Leu Pro Tyr Gly  
 100 105 110  
 Ala Val Ala His Glu Ala Phe Leu Tyr Phe Leu Ser Tyr Ile Tyr Thr  
 115 120 125  
 Gly Arg Leu Lys Pro Phe Pro Leu Glu Val Ser Thr Cys Val Asp Pro

130	135	140
Val Cys Ser His Asp Cys Cys Arg Pro Ala Ile Asp Phe Val Val Gln 145 150 155 160		
Leu Met Tyr Ala Ser Ser Val Leu Gln Val Pro Glu Leu Val Ser Ser 165 170 175		
Phe Gln Arg Arg Leu Cys Asn Phe Val Glu Lys Thr Leu Val Glu Asn 180 185 190		
Val Leu Pro Ile Leu Met Val Ala Phe Asn Cys Lys Leu Thr Gln Leu 195 200 205		
Leu Asp Gln Cys Ile Glu Arg Val Ala Arg Ser Asp Leu Tyr Arg Phe 210 215 220		
Cys Ile Glu Lys Glu Val Pro Pro Glu Val Ala Glu Lys Ile Lys Gln 225 230 235 240		
Leu Arg Leu Ile Ser Pro Gln Asp Glu Glu Thr Ser Pro Lys Ile Ser 245 250 255		
Glu Lys Leu Leu Glu Arg Ile Gly Lys Ile Leu Lys Ala Leu Asp Ser 260 265 270		
Asp Asp Val Glu Leu Val Lys Leu Leu Leu Thr Glu Ser Asp Ile Thr 275 280 285		
Leu Asp Gln Ala Asn Gly Leu His Tyr Ser Val Val Tyr Ser Asp Pro 290 295 300		
Lys Val Val Ala Glu Ile Leu Ala Leu Asp Met Gly Asp Val Asn Tyr 305 310 315 320		
Arg Asn Ser Arg Gly Tyr Thr Val Leu His Phe Ala Ala Met Arg Arg 325 330 335		
Glu Pro Ser Ile Ile Ile Ser Leu Ile Asp Lys Gly Ala Asn Ala Ser 340 345 350		
Glu Phe Thr Ser Asp Gly Arg Ser Ala Val Asn Ile Leu Arg Arg Leu 355 360 365		
Thr Asn Pro Lys Asp Tyr His Thr Lys Thr Ala Lys Gly Arg Glu Ser 370 375 380		
Ser Lys Ala Arg Leu Cys Ile Asp Ile Leu Glu Arg Glu Ile Arg Lys 385 390 395 400		
Asn Pro Met Val Leu Asp Thr Pro Met Cys Ser Ile Ser Met Pro Glu 405 410 415		
Asp Leu Gln Met Arg Leu Leu Tyr Leu Glu Lys Arg Val Gly Leu Ala 420 425 430		
Gln Leu Phe Phe Pro Thr Glu Ala Lys Val Ala Met Asp Ile Gly Asn 435 440 445		
Val Glu Gly Thr Ser Glu Phe Thr Gly Leu Ser Pro Pro Ser Ser Gly 450 455 460		



Leu Thr Gly Asn Leu Ser Gln Val Asp Leu Asn Glu Thr Pro His Met  
 465 470 475 480  
 Gln Thr Gln Arg Leu Leu Thr Arg Met Val Ala Leu Met Lys Thr Val  
 485 490 495  
 Glu Thr Gly Arg Arg Phe Phe Pro Tyr Gly Ser Glu Val Leu Asp Lys  
 500 505 510  
 Tyr Met Ala Glu Tyr Ile Asp Asp Asp Ile Leu Asp Asp Phe His Phe  
 515 520 525  
 Glu Lys Gly Ser Thr His Glu Arg Arg Leu Lys Arg Met Arg Tyr Arg  
 530 535 540  
 Glu Leu Lys Asp Asp Val Gln Lys Ala Tyr Ser Lys Asp Lys Glu Ser  
 545 550 555 560  
 Lys Ile Ala Arg Ser Cys Leu Ser Ala Ser Ser Ser Pro Ser Ser Ser  
 565 570 575  
 Ser Ile Arg Asp Asp Leu His Asn Thr Thr  
 580 585

<210> 9  
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 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR Primer

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<210> 10  
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 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR Primer

<400> 10  
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<210> 11  
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 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR Primer

<400> 11  
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<210> 12
<211> 23
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer

<400> 12
gcggatccta tttcctaaaa ggg                23

<210> 13
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer

<400> 13
tcaaggcctt ggattcagat g                21

<210> 14
<211> 21
<212> DNA
<213> Artificial Sequence

<220>
<223> Description of Artificial Sequence: PCR Primer

<400> 14
attaactgcg ctacgtccgt c                21

<210> 15
<211> 1477
<212> DNA
<213> Arabidopsis thaliana

<220>
<221> CDS
<222> (1)..(1476)
<223> AtNMLc2 genomic sequence

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Met Ser Asn Leu Glu Ser Leu Arg Ser Leu Ser Leu Asp Phe Leu
   1             5             10             15

aac cta cta atc aac ggt caa gct ttc tcc gac gtg act ttc agc gtt    96
Asn Leu Leu Ile Asn Gly Gln Ala Phe Ser Asp Val Thr Phe Ser Val
   20             25             30

gaa ggt cgt tta gtc cac gct cac cgt tgt atc ctc gcc gca cgg agt    144
Glu Gly Arg Leu Val His Ala His Arg Cys Ile Leu Ala Ala Arg Ser
   35             40             45

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ctt ttc ttc cgc aaa ttc ttt tgt ggg aca gac tca cca caa cct gtc	192
Leu Phe Phe Arg Lys Phe Phe Cys Gly Thr Asp Ser Pro Gln Pro Val	
50 55 60	
aca ggt ata gac ccg acc caa cat ggg tcc gta ccc gct agc cca aca	240
Thr Gly Ile Asp Pro Thr Gln His Gly Ser Val Pro Ala Ser Pro Thr	
65 70 75 80	
aga ggc tcc acg gcc cca gct gga att ata cca gtg aac tca gtc ggt	288
Arg Gly Ser Thr Ala Pro Ala Gly Ile Ile Pro Val Asn Ser Val Gly	
85 90 95	
tat gag gtt ttt ctg ttg cta ctt cag ttt ctt tat agc gga caa gtc	336
Tyr Glu Val Phe Leu Leu Leu Leu Gln Phe Leu Tyr Ser Gly Gln Val	
100 105 110	
tcc atc gtg ccg cag aaa cac gag cct aga cct aat tgt ggc gag aga	384
Ser Ile Val Pro Gln Lys His Glu Pro Arg Pro Asn Cys Gly Glu Arg	
115 120 125	
gga tgt tgg cac act cat tgc tca gcc gcc gtt gat ctt gct ctt gat	432
Gly Cys Trp His Thr His Cys Ser Ala Ala Val Asp Leu Ala Leu Asp	
130 135 140	
act ctc gcc gcc tct cgt tac ttc ggc gtc gag cag ctc gca ttg ctc	480
Thr Leu Ala Ala Ser Arg Tyr Phe Gly Val Glu Gln Leu Ala Leu Leu	
145 150 155 160	
acc cag aaa caa ttg gca agc atg gtg gag aaa gcc tct atc gaa gat	528
Thr Gln Lys Gln Leu Ala Ser Met Val Glu Lys Ala Ser Ile Glu Asp	
165 170 175	
gtg atg aaa gtt tta ata gca tca aga aag caa gac atg cat caa tta	576
Val Met Lys Val Leu Ile Ala Ser Arg Lys Gln Asp Met His Gln Leu	
180 185 190	
tgg acc acc tgc tct cac tta gtt atg agc aat ctt gaa gaa tct ttg	624
Trp Thr Thr Cys Ser His Leu Val Met Ser Asn Leu Glu Glu Ser Leu	
195 200 205	
aga tct cta tcg ttg gat ttc ctg aac cta cta atc aac ggt caa gct	672
Arg Ser Leu Ser Leu Asp Phe Leu Asn Leu Leu Ile Asn Gly Gln Ala	
210 215 220	
ttc tcc gac gtg act ttc agc gtt gaa ggt cgt tta gtc cac gct cac	720
Phe Ser Asp Val Thr Phe Ser Val Glu Gly Arg Leu Val His Ala His	
225 230 235 240	
cgt tgt atc ctc gcc gca cgg agt ctt ttc ttc cgc aaa ttc ttt tgt	768
Arg Cys Ile Leu Ala Ala Arg Ser Leu Phe Phe Arg Lys Phe Phe Cys	
245 250 255	
ggg aca gac tca cca caa cct gtc aca ggt ata gac ccg acc caa cat	816
Gly Thr Asp Ser Pro Gln Pro Val Thr Gly Ile Asp Pro Thr Gln His	
260 265 270	
ggg tcc gta ccc gct agc cca aca aga ggc tcc acg gcc cca gct gga	864
Gly Ser Val Pro Ala Ser Pro Thr Arg Gly Ser Thr Ala Pro Ala Gly	
275 280 285	
att ata cca gtg aac tca gtc ggt tat gag gtt ttt ctg ttg cta ctt	912

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Ile Ile Pro Val Asn Ser Val Gly Tyr Glu Val Phe Leu Leu Leu Leu
290                               295                               300

cag ttt ctt tat agc gga caa gtc tcc atc gtg ccg cag aaa cac gag 960
Gln Phe Leu Tyr Ser Gly Gln Val Ser Ile Val Pro Gln Lys His Glu
305                               310                               315                               320

cct aga cct aat tgt ggc gag aga gga tgt tgg cac act cat tgc tca 1008
Pro Arg Pro Asn Cys Gly Glu Arg Gly Cys Trp His Thr His Cys Ser
325                               330                               335

gcc gcc gtt gat ctt gct ctt gat act ctc gcc gcc tct cgt tac ttc 1056
Ala Ala Val Asp Leu Ala Leu Asp Thr Leu Ala Ala Ser Arg Tyr Phe
340                               345                               350

ggc gtc gag cag ctc gca ttg ctc acc cag aaa caa ttg gca agc atg 1104
Gly Val Glu Gln Leu Ala Leu Leu Thr Gln Lys Gln Leu Ala Ser Met
355                               360                               365

gtg gag aaa gcc tct atc gaa gat gtg atg aaa gtt tta ata gca tca 1152
Val Glu Lys Ala Ser Ile Glu Asp Val Met Lys Val Leu Ile Ala Ser
370                               375                               380

aga aag caa gac atg cat caa tta tgg acc acc tgc tct cac tta gtt 1200
Arg Lys Gln Asp Met His Gln Leu Trp Thr Thr Cys Ser His Leu Val
385                               390                               395                               400

atg agc aat ctt gaa gaa tct ttg aga tct cta tcg ttg gat ttc ctg 1248
Met Ser Asn Leu Glu Glu Ser Leu Arg Ser Leu Ser Leu Asp Phe Leu
405                               410                               415

aac cta cta atc aac ggt caa gct ttc tcc gac gtg act ttc agc gtt 1296
Asn Leu Leu Ile Asn Gly Gln Ala Phe Ser Asp Val Thr Phe Ser Val
420                               425                               430

gaa ggt cgt tta gtc cac gct cac cgt tgt atc ctc gcc gca cgg agt 1344
Glu Gly Arg Leu Val His Ala His Arg Cys Ile Leu Ala Ala Arg Ser
435                               440                               445

ctt ttc ttc cgc aaa ttc ttt tgt ggg aca gac tca cca caa cct gtc 1392
Leu Phe Phe Arg Lys Phe Phe Cys Gly Thr Asp Ser Pro Gln Pro Val
450                               455                               460

aca ggt ata gac ccg acc caa cat ggg tcc gta ccc gct agc cca aca 1440
Thr Gly Ile Asp Pro Thr Gln His Gly Ser Val Pro Ala Ser Pro Thr
465                               470                               475                               480

aga ggc tcc acg gcc cca gct gga att ata cca gtg a 1477
Arg Gly Ser Thr Ala Pro Ala Gly Ile Ile Pro Val
485                               490

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&lt;210&gt; 16

&lt;211&gt; 492

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 16

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Met Ser Asn Leu Glu Glu Ser Leu Arg Ser Leu Ser Leu Asp Phe Leu
1                               5                               10                               15

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 20 25 30  
 Glu Gly Arg Leu Val His Ala His Arg Cys Ile Leu Ala Ala Arg Ser  
 35 40 45  
 Leu Phe Phe Arg Lys Phe Phe Cys Gly Thr Asp Ser Pro Gln Pro Val  
 50 55 60  
 Thr Gly Ile Asp Pro Thr Gln His Gly Ser Val Pro Ala Ser Pro Thr  
 65 70 75 80  
 Arg Gly Ser Thr Ala Pro Ala Gly Ile Ile Pro Val Asn Ser Val Gly  
 85 90 95  
 Tyr Glu Val Phe Leu Leu Leu Leu Gln Phe Leu Tyr Ser Gly Gln Val  
 100 105 110  
 Ser Ile Val Pro Gln Lys His Glu Pro Arg Pro Asn Cys Gly Glu Arg  
 115 120 125  
 Gly Cys Trp His Thr His Cys Ser Ala Ala Val Asp Leu Ala Leu Asp  
 130 135 140  
 Thr Leu Ala Ala Ser Arg Tyr Phe Gly Val Glu Gln Leu Ala Leu Leu  
 145 150 155 160  
 Thr Gln Lys Gln Leu Ala Ser Met Val Glu Lys Ala Ser Ile Glu Asp  
 165 170 175  
 Val Met Lys Val Leu Ile Ala Ser Arg Lys Gln Asp Met His Gln Leu  
 180 185 190  
 Trp Thr Thr Cys Ser His Leu Val Met Ser Asn Leu Glu Glu Ser Leu  
 195 200 205  
 Arg Ser Leu Ser Leu Asp Phe Leu Asn Leu Leu Ile Asn Gly Gln Ala  
 210 215 220  
 Phe Ser Asp Val Thr Phe Ser Val Glu Gly Arg Leu Val His Ala His  
 225 230 235 240  
 Arg Cys Ile Leu Ala Ala Arg Ser Leu Phe Phe Arg Lys Phe Phe Cys  
 245 250 255  
 Gly Thr Asp Ser Pro Gln Pro Val Thr Gly Ile Asp Pro Thr Gln His  
 260 265 270  
 Gly Ser Val Pro Ala Ser Pro Thr Arg Gly Ser Thr Ala Pro Ala Gly  
 275 280 285  
 Ile Ile Pro Val Asn Ser Val Gly Tyr Glu Val Phe Leu Leu Leu Leu  
 290 295 300  
 Gln Phe Leu Tyr Ser Gly Gln Val Ser Ile Val Pro Gln Lys His Glu  
 305 310 315 320  
 Pro Arg Pro Asn Cys Gly Glu Arg Gly Cys Trp His Thr His Cys Ser  
 325 330 335  
 Ala Ala Val Asp Leu Ala Leu Asp Thr Leu Ala Ala Ser Arg Tyr Phe

	340		345		350	
Gly Val Glu Gln Leu Ala Leu Leu Thr Gln Lys Gln Leu Ala Ser Met	355		360		365	
Val Glu Lys Ala Ser Ile Glu Asp Val Met Lys Val Leu Ile Ala Ser	370		375		380	
Arg Lys Gln Asp Met His Gln Leu Trp Thr Thr Cys Ser His Leu Val	385		390		395	400
Met Ser Asn Leu Glu Glu Ser Leu Arg Ser Leu Ser Leu Asp Phe Leu		405		410		415
Asn Leu Leu Ile Asn Gly Gln Ala Phe Ser Asp Val Thr Phe Ser Val		420		425		430
Glu Gly Arg Leu Val His Ala His Arg Cys Ile Leu Ala Ala Arg Ser		435		440		445
Leu Phe Phe Arg Lys Phe Phe Cys Gly Thr Asp Ser Pro Gln Pro Val		450		455		460
Thr Gly Ile Asp Pro Thr Gln His Gly Ser Val Pro Ala Ser Pro Thr		465		470		475
		475		480		
Arg Gly Ser Thr Ala Pro Ala Gly Ile Ile Pro Val		485		490		

<210> 17  
 <211> 1804  
 <212> DNA  
 <213> Arabidopsis thaliana  
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 <222> (1)..(1803)  
 <223> AtNMLc4-1 genomic sequence

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1 5 10 15	
tct cac tta tca aac cct tct cct gtt gtt act act tat cac tca gct	96
Ser His Leu Ser Asn Pro Ser Pro Val Val Thr Thr Tyr His Ser Ala	
20 25 30	
gct aat ctt gaa gag ctc agc tct aac ttg gag cag ctt ctc act aat	144
Ala Asn Leu Glu Glu Leu Ser Ser Asn Leu Glu Gln Leu Leu Thr Asn	
35 40 45	
cca gat tgc gat tac act gac gca gag atc atc att gaa gaa gaa gct	192
Pro Asp Cys Asp Tyr Thr Asp Ala Glu Ile Ile Ile Glu Glu Glu Ala	
50 55 60	
aac cct gtg agt gtt cat aga tgt gtt tta gct gct agg agc aag ttt	240
Asn Pro Val Ser Val His Arg Cys Val Leu Ala Ala Arg Ser Lys Phe	
65 70 75 80	

ttt ctt gat ctg ttt aag aaa gat aaa gat agt agt gag aag aaa cct	288
Phe Leu Asp Leu Phe Lys Lys Asp Lys Asp Ser Ser Glu Lys Lys Pro	
85 90 95	
aag tat caa atg aaa gat tta tta cca tat gga aat gtg gga cgt gag	336
Lys Tyr Gln Met Lys Asp Leu Leu Pro Tyr Gly Asn Val Gly Arg Glu	
100 105 110	
gca ttt ctg cat ttc ttg agc tat atc tac act ggg agg tta aag cct	384
Ala Phe Leu His Phe Leu Ser Tyr Ile Tyr Thr Gly Arg Leu Lys Pro	
115 120 125	
ttt cct atc gag gtt tca act tgt gtt gat tca gtt tgt gct cat gat	432
Phe Pro Ile Glu Val Ser Thr Cys Val Asp Ser Val Cys Ala His Asp	
130 135 140	
tct tgt aaa ccg gcc att gat ttt gct gtt gag ttg atg tat gct tca	480
Ser Cys Lys Pro Ala Ile Asp Phe Ala Val Glu Leu Met Tyr Ala Ser	
145 150 155 160	
ttt gtg ttc caa atc ccg gat ctt gtt tcg tca ttt cag ccg aag ctt	528
Phe Val Phe Gln Ile Pro Asp Leu Val Ser Ser Phe Gln Arg Lys Leu	
165 170 175	
cgt aac tat gtt gag aag tca cta gta gag aat gtt ctt cct atc ctc	576
Arg Asn Tyr Val Glu Lys Ser Leu Val Glu Asn Val Leu Pro Ile Leu	
180 185 190	
tta gtt gcg ttt cat tgt gat ttg aca cag ctt ctt gat caa tgc att	624
Leu Val Ala Phe His Cys Asp Leu Thr Gln Leu Leu Asp Gln Cys Ile	
195 200 205	
gag aga gtg gcg aga tca gac tta gac aga ttc tgt atc gaa aag gag	672
Glu Arg Val Ala Arg Ser Asp Leu Asp Arg Phe Cys Ile Glu Lys Glu	
210 215 220	
ctt cct tta gaa gta ttg gaa aaa atc aaa cag ctt cga gtt aag tcg	720
Leu Pro Leu Glu Val Leu Glu Lys Ile Lys Gln Leu Arg Val Lys Ser	
225 230 235 240	
gtg aac ata ccc gag gtg gag gat aaa tcg ata gag aga aca ggg aaa	768
Val Asn Ile Pro Glu Val Glu Asp Lys Ser Ile Glu Arg Thr Gly Lys	
245 250 255	
gta ctc aag gca ttg gat tca gat gat gta gaa ctc gtg aag ctt ctt	816
Val Leu Lys Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu	
260 265 270	
ttg act gag tca gat ata act cta gac caa gcc aat ggt cta cat tat	864
Leu Thr Glu Ser Asp Ile Thr Leu Asp Gln Ala Asn Gly Leu His Tyr	
275 280 285	
gca gtg gca tac agt gat ccg aaa gtt gtg aca cag gtt ctt gat cta	912
Ala Val Ala Tyr Ser Asp Pro Lys Val Val Thr Gln Val Leu Asp Leu	
290 295 300	
gat atg gct gat gtt aat ttc aga aat tcc agg ggg tat acg gtt ctt	960
Asp Met Ala Asp Val Asn Phe Arg Asn Ser Arg Gly Tyr Thr Val Leu	
305 310 315 320	

cat att gct gct atg cgt aga gag cca aca att atc ata cca ctt att	1008
His Ile Ala Ala Met Arg Arg Glu Pro Thr Ile Ile Ile Pro Leu Ile	
325 330 335	
caa aaa gga gct aat gct tca gat ttc acg ttt gat gga cgc agt gcg	1056
Gln Lys Gly Ala Asn Ala Ser Asp Phe Thr Phe Asp Gly Arg Ser Ala	
340 345 350	
gta aat ata tgt agg aga ctc act agg ccg aaa gat tat cat acc aaa	1104
Val Asn Ile Cys Arg Arg Leu Thr Arg Pro Lys Asp Tyr His Thr Lys	
355 360 365	
acc tca agg aaa gaa cct agt aaa tac cgc tta tgc atc gat atc ttg	1152
Thr Ser Arg Lys Glu Pro Ser Lys Tyr Arg Leu Cys Ile Asp Ile Leu	
370 375 380	
gaa agg gaa att aga agg aat cca ttg gtt agt ggg gat aca ccc act	1200
Glu Arg Glu Ile Arg Arg Asn Pro Leu Val Ser Gly Asp Thr Pro Thr	
385 390 395 400	
tgt tcc cat tcg atg ccc gag gat ctc caa atg agg ttg tta tac tta	1248
Cys Ser His Ser Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu	
405 410 415	
gaa aag cga tgg gac ttg cgt cag ttg ttc ttc cca gca gaa gcc aat	1296
Glu Lys Arg Trp Asp Leu Arg Gln Leu Phe Phe Pro Ala Glu Ala Asn	
420 425 430	
gtg gct atg gac gtt gct aat gtt gaa ggg aca agc gag tgc aca ggt	1344
Val Ala Met Asp Val Ala Asn Val Glu Gly Thr Ser Glu Cys Thr Gly	
435 440 445	
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Leu Leu Thr Pro Pro Pro Ser Asn Asp Thr Thr Thr Asn Leu Gly Lys	
450 455 460	
gtc gat tta aat gaa acg cct tat gtg caa acg aaa aga atg ctt aca	1440
Val Asp Leu Asn Glu Thr Pro Tyr Val Gln Thr Lys Arg Met Leu Thr	
465 470 475 480	
cgt atg aaa gcc ctc atg aaa aca ggt aaa agc tta agg aaa tgt act	1488
Arg Met Lys Ala Leu Met Lys Thr Gly Lys Ser Leu Arg Lys Cys Thr	
485 490 495	
ttc aag ttt tat tct ctg acc aca aga ttg act gat tcg aaa ccg ttc	1536
Phe Lys Phe Tyr Ser Leu Thr Thr Arg Leu Thr Asp Ser Lys Pro Phe	
500 505 510	
aac aac gca gtt gag aca ggt cgg aga tac ttc cca tct tgt tat gag	1584
Asn Asn Ala Val Glu Thr Gly Arg Arg Tyr Phe Pro Ser Cys Tyr Glu	
515 520 525	
gtt ctg gat aag tac atg gat cag tat atg gac gaa gaa atc cct gat	1632
Val Leu Asp Lys Tyr Met Asp Gln Tyr Met Asp Glu Glu Ile Pro Asp	
530 535 540	
atg tcg tat ccc gag aaa ggc act gtg aaa gag aga aga cag aag agg	1680
Met Ser Tyr Pro Glu Lys Gly Thr Val Lys Glu Arg Arg Gln Lys Arg	
545 550 555 560	
atg aga tat aac gag ctg aag aac gac gtt aaa aaa gca tat agc aaa	1728



Met Arg Tyr Asn Glu Leu Lys Asn Asp Val Lys Lys Ala Tyr Ser Lys  
565 570 575

gac aaa gtc gcg cgg tct tgt ctt tct tct tca tca cca gct tct tct 1776  
Asp Lys Val Ala Arg Ser Cys Leu Ser Ser Ser Ser Pro Ala Ser Ser  
580 585 590

ctt aga gaa gcc tta gag aat cca aca t 1804  
Leu Arg Glu Ala Leu Glu Asn Pro Thr  
595 600

<210> 18  
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<212> PRT  
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Ala Asn Leu Glu Glu Leu Ser Ser Asn Leu Glu Gln Leu Leu Thr Asn  
35 40 45

Pro Asp Cys Asp Tyr Thr Asp Ala Glu Ile Ile Ile Glu Glu Glu Ala  
50 55 60

Asn Pro Val Ser Val His Arg Cys Val Leu Ala Ala Arg Ser Lys Phe  
65 70 75 80

Phe Leu Asp Leu Phe Lys Lys Asp Lys Asp Ser Ser Glu Lys Lys Pro  
85 90 95

Lys Tyr Gln Met Lys Asp Leu Leu Pro Tyr Gly Asn Val Gly Arg Glu  
100 105 110

Ala Phe Leu His Phe Leu Ser Tyr Ile Tyr Thr Gly Arg Leu Lys Pro  
115 120 125

Phe Pro Ile Glu Val Ser Thr Cys Val Asp Ser Val Cys Ala His Asp  
130 135 140

Ser Cys Lys Pro Ala Ile Asp Phe Ala Val Glu Leu Met Tyr Ala Ser  
145 150 155 160

Phe Val Phe Gln Ile Pro Asp Leu Val Ser Ser Phe Gln Arg Lys Leu  
165 170 175

Arg Asn Tyr Val Glu Lys Ser Leu Val Glu Asn Val Leu Pro Ile Leu  
180 185 190

Leu Val Ala Phe His Cys Asp Leu Thr Gln Leu Leu Asp Gln Cys Ile  
195 200 205

Glu Arg Val Ala Arg Ser Asp Leu Asp Arg Phe Cys Ile Glu Lys Glu  
210 215 220

Leu Pro Leu Glu Val Leu Glu Lys Ile Lys Gln Leu Arg Val Lys Ser

225		230		235		240
Val Asn Ile Pro	Glu Val Glu Asp Lys Ser Ile Glu Arg Thr Gly Lys					
	245			250		255
Val Leu Lys Ala	Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu					
	260		265			270
Leu Thr Glu Ser	Asp Ile Thr Leu Asp Gln Ala Asn Gly Leu His Tyr					
	275		280			285
Ala Val Ala Tyr	Ser Asp Pro Lys Val Val Thr Gln Val Leu Asp Leu					
	290		295			300
Asp Met Ala Asp	Val Asn Phe Arg Asn Ser Arg Gly Tyr Thr Val Leu					
	305		310		315	320
His Ile Ala Ala	Met Arg Arg Glu Pro Thr Ile Ile Ile Pro Leu Ile					
	325		330			335
Gln Lys Gly Ala	Asn Ala Ser Asp Phe Thr Phe Asp Gly Arg Ser Ala					
	340		345			350
Val Asn Ile Cys	Arg Arg Leu Thr Arg Pro Lys Asp Tyr His Thr Lys					
	355		360			365
Thr Ser Arg Lys	Glu Pro Ser Lys Tyr Arg Leu Cys Ile Asp Ile Leu					
	370		375			380
Glu Arg Glu Ile	Arg Arg Asn Pro Leu Val Ser Gly Asp Thr Pro Thr					
	385		390		395	400
Cys Ser His Ser	Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu					
	405		410			415
Glu Lys Arg Trp	Asp Leu Arg Gln Leu Phe Phe Pro Ala Glu Ala Asn					
	420		425			430
Val Ala Met Asp	Val Ala Asn Val Glu Gly Thr Ser Glu Cys Thr Gly					
	435		440			445
Leu Leu Thr Pro	Pro Pro Ser Asn Asp Thr Thr Glu Asn Leu Gly Lys					
	450		455			460
Val Asp Leu Asn	Glu Thr Pro Tyr Val Gln Thr Lys Arg Met Leu Thr					
	465		470		475	480
Arg Met Lys Ala	Leu Met Lys Thr Gly Lys Ser Leu Arg Lys Cys Thr					
	485		490			495
Phe Lys Phe Tyr	Ser Leu Thr Thr Arg Leu Thr Asp Ser Lys Pro Phe					
	500		505			510
Asn Asn Ala Val	Glu Thr Gly Arg Arg Tyr Phe Pro Ser Cys Tyr Glu					
	515		520			525
Val Leu Asp Lys	Tyr Met Asp Gln Tyr Met Asp Glu Glu Ile Pro Asp					
	530		535			540
Met Ser Tyr Pro	Glu Lys Gly Thr Val Lys Glu Arg Arg Gln Lys Arg					
	545		550		555	560

Met Arg Tyr Asn Glu Leu Lys Asn Asp Val Lys Lys Ala Tyr Ser Lys  
 565 570 575  
 Asp Lys Val Ala Arg Ser Cys Leu Ser Ser Ser Ser Pro Ala Ser Ser  
 580 585 590  
 Leu Arg Glu Ala Leu Glu Asn Pro Thr  
 595 600

<210> 19  
 <211> 1803  
 <212> DNA  
 <213> Arabidopsis thaliana

<220>  
 <221> CDS  
 <222> (1)..(1803)  
 <223> AtNMLc4-2 genomic sequence

<400> 19  
 atg gcc acc acc acc acc acc acc acc gct aga ttc tct gat tca tac 48  
 Met Ala Thr Thr Thr Thr Thr Thr Thr Ala Arg Phe Ser Asp Ser Tyr  
 1 5 10 15  
 gag ttc agc aac aca agc ggc aat agc ttc ttc gcc gcc gag tca tct 96  
 Glu Phe Ser Asn Thr Ser Gly Asn Ser Phe Phe Ala Ala Glu Ser Ser  
 20 25 30  
 ctt gat tat ccg acg gaa ttt ctc acg cca ccg gag gta tca gct ctt 144  
 Leu Asp Tyr Pro Thr Glu Phe Leu Thr Pro Pro Glu Val Ser Ala Leu  
 35 40 45  
 aaa ctt ctg tct aac tgc ctc gag tct gtt ttc gac tcg ccg gag acg 192  
 Lys Leu Leu Ser Asn Cys Leu Glu Ser Val Phe Asp Ser Pro Glu Thr  
 50 55 60  
 ttc tac agc gat gct aag cta gtt ctc gcc gcc gcc cgg gaa gtt tct 240  
 Phe Tyr Ser Asp Ala Lys Leu Val Leu Ala Gly Gly Arg Glu Val Ser  
 65 70 75 80  
 ttt cac cgt tgt att ctt tcc gcg aga att cct gtc ttc aaa agc gct 288  
 Phe His Arg Cys Ile Leu Ser Ala Arg Ile Pro Val Phe Lys Ser Ala  
 85 90 95  
 tta gcc acc gtg aag gaa caa aaa tcc tcc acc acc gtg aag ctc cag 336  
 Leu Ala Thr Val Lys Glu Gln Lys Ser Ser Thr Thr Val Lys Leu Gln  
 100 105 110  
 ctg aaa gag atc gcc aga gat tac gaa gtc gcc ttt gac tcg gtt gtg 384  
 Leu Lys Glu Ile Ala Arg Asp Tyr Glu Val Gly Phe Asp Ser Val Val  
 115 120 125  
 gcg gtt ttg gcg tat gtt tac agc gcc aga gtg agg tcc ccg ccg aag 432  
 Ala Val Leu Ala Tyr Val Tyr Ser Gly Arg Val Arg Ser Pro Pro Lys  
 130 135 140  
 gga gct tct gct tgc gta gac gac gat tgt tgc cac gtg gct tgc ccg 480  
 Gly Ala Ser Ala Cys Val Asp Asp Asp Cys Cys His Val Ala Cys Arg

145	150	155	160	
tca aag gtg gat ttc atg gtg gag gtt ctt tat ctg tct ttc gtt ttc	528			
Ser Lys Val Asp Phe Met Val Glu Val Leu Tyr Leu Ser Phe Val Phe				
165	170	175		
cag att caa gaa tta gtt act ctg tat gag agg cag ttc ttg gaa att	576			
Gln Ile Gln Glu Leu Val Thr Leu Tyr Glu Arg Gln Phe Leu Glu Ile				
180	185	190		
gta gac aaa gtt gta gtc gaa gac atc ttg gtt ata ttc aag ctt gat	624			
Val Asp Lys Val Val Val Glu Asp Ile Leu Val Ile Phe Lys Leu Asp				
195	200	205		
act cta tgt ggt aca aca tac aag aag ctt ttg gat aga tgc ata gaa	672			
Thr Leu Cys Gly Thr Thr Tyr Lys Lys Leu Leu Asp Arg Cys Ile Glu				
210	215	220		
att atc gtg aag tct gat ata gaa cta gtt agt ctt gag aag tct tta	720			
Ile Ile Val Lys Ser Asp Ile Glu Leu Val Ser Leu Glu Lys Ser Leu				
225	230	235	240	
cct caa cac att ttc aag caa atc ata gac atc cgc gaa gcg ctc tgt	768			
Pro Gln His Ile Phe Lys Gln Ile Ile Asp Ile Arg Glu Ala Leu Cys				
245	250	255		
cta gag cca cct aaa cta gaa agg cat gtc aag aac ata tac aag gcg	816			
Leu Glu Pro Pro Lys Leu Glu Arg His Val Lys Asn Ile Tyr Lys Ala				
260	265	270		
cta gac tca gat gat gtt gag ctt gtc aag atg ctt ttg cta gaa gga	864			
Leu Asp Ser Asp Asp Val Glu Leu Val Lys Met Leu Leu Leu Glu Gly				
275	280	285		
cac acc aat ctc gat gag gcg tat gct ctt cat ttt gct atc gct cac	912			
His Thr Asn Leu Asp Glu Ala Tyr Ala Leu His Phe Ala Ile Ala His				
290	295	300		
tgc gct gtg aag acc gcg tat gat ctc ctc gag ctt gag ctt gcg gat	960			
Cys Ala Val Lys Thr Ala Tyr Asp Leu Leu Glu Leu Glu Leu Ala Asp				
305	310	315	320	
gtt aac ctt aga aat ccg agg gga tac act gtg ctt cat gtt gct gcg	1008			
Val Asn Leu Arg Asn Pro Arg Gly Tyr Thr Val Leu His Val Ala Ala				
325	330	335		
atg cgg aag gag ccg aag ttg ata ata tct ttg tta atg aaa ggg gca	1056			
Met Arg Lys Glu Pro Lys Leu Ile Ile Ser Leu Leu Met Lys Gly Ala				
340	345	350		
aat att tta gac aca aca ttg gat ggt aga acc gct tta gtg att gta	1104			
Asn Ile Leu Asp Thr Thr Leu Asp Gly Arg Thr Ala Leu Val Ile Val				
355	360	365		
aaa cga ctc act aaa gcg gat gac tac aaa act agt acg gag gac ggt	1152			
Lys Arg Leu Thr Lys Ala Asp Asp Tyr Lys Thr Ser Thr Glu Asp Gly				
370	375	380		
acg cct tct ctg aaa ggc gga tta tgc ata gag gta ctt gag cat gaa	1200			
Thr Pro Ser Leu Lys Gly Gly Leu Cys Ile Glu Val Leu Glu His Glu				
385	390	395	400	

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caa aaa cta gaa tat ttg tgc cct ata gag gct tca ctt tct ctt cca 1248
Gln Lys Leu Glu Tyr Leu Ser Pro Ile Glu Ala Ser Leu Ser Leu Pro
      405      410      415

gta act cca gag gag ttg agg atg agg ttg ctc tat tat gaa aac cga 1296
Val Thr Pro Glu Glu Leu Arg Met Arg Leu Leu Tyr Tyr Glu Asn Arg
      420      425      430

gtt gca ctt gct cga ctt ctc ttt cca gtg gaa act gaa act gta cag 1344
Val Ala Leu Ala Arg Leu Leu Phe Pro Val Glu Thr Glu Thr Val Gln
      435      440      445

ggt att gcc aaa ttg gag gaa aca tgc gag ttt aca gct tct agt ctc 1392
Gly Ile Ala Lys Leu Glu Glu Thr Cys Glu Phe Thr Ala Ser Ser Leu
      450      455      460

gag cct gat cat cac att ggt gaa aag cgg aca tca cta gac cta aat 1440
Glu Pro Asp His His Ile Gly Glu Lys Arg Thr Ser Leu Asp Leu Asn
      465      470      475      480

atg gcg ccg ttc caa atc cat gag aag cat ttg agt aga cta aga gca 1488
Met Ala Pro Phe Gln Ile His Glu Lys His Leu Ser Arg Leu Arg Ala
      485      490      495

ctt tgt aaa acc gtg gaa ctg ggg aaa cgc tac ttc aaa cga tgt tcg 1536
Leu Cys Lys Thr Val Glu Leu Gly Lys Arg Tyr Phe Lys Arg Cys Ser
      500      505      510

ctt gat cac ttt atg gat act gag gac ttg aat cat ctt gct agc gta 1584
Leu Asp His Phe Met Asp Thr Glu Asp Leu Asn His Leu Ala Ser Val
      515      520      525

gaa gaa gat act cct gag aaa cgg cta caa aag aag caa agg tac atg 1632
Glu Glu Asp Thr Pro Glu Lys Arg Leu Gln Lys Lys Gln Arg Tyr Met
      530      535      540

gaa cta caa gag act ctg atg aag acc ttt agt gag gac aag gag gaa 1680
Glu Leu Gln Glu Thr Leu Met Lys Thr Phe Ser Glu Asp Lys Glu Glu
      545      550      555      560

tgt gga aag tct tcc aca ccg aaa cca acc tct gcg gtg agg tct aat 1728
Cys Gly Lys Ser Ser Thr Pro Lys Pro Thr Ser Ala Val Arg Ser Asn
      565      570      575

aga aaa ctc tct cac cgg cgc cta aaa gtg gac aaa cgg gat ttt ttg 1776
Arg Lys Leu Ser His Arg Arg Leu Lys Val Asp Lys Arg Asp Phe Leu
      580      585      590

aaa cga cct tac ggg aac ggg gat taa 1803
Lys Arg Pro Tyr Gly Asn Gly Asp
      595      600

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&lt;210&gt; 20

&lt;211&gt; 600

&lt;212&gt; PRT

&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 20

Met Ala Thr Thr Thr Thr Thr Thr Thr Ala Arg Phe Ser Asp Ser Tyr

1	5					10					15				
Glu	Phe	Ser	Asn	Thr	Ser	Gly	Asn	Ser	Phe	Phe	Ala	Ala	Glu	Ser	Ser
Leu	Asp	Tyr	Pro	Thr	Glu	Phe	Leu	Thr	Pro	Pro	Glu	Val	Ser	Ala	Leu
Lys	Leu	Leu	Ser	Asn	Cys	Leu	Glu	Ser	Val	Phe	Asp	Ser	Pro	Glu	Thr
Phe	Tyr	Ser	Asp	Ala	Lys	Leu	Val	Leu	Ala	Gly	Gly	Arg	Glu	Val	Ser
Phe	His	Arg	Cys	Ile	Leu	Ser	Ala	Arg	Ile	Pro	Val	Phe	Lys	Ser	Ala
Leu	Ala	Thr	Val	Lys	Glu	Gln	Lys	Ser	Ser	Thr	Thr	Val	Lys	Leu	Gln
Leu	Lys	Glu	Ile	Ala	Arg	Asp	Tyr	Glu	Val	Gly	Phe	Asp	Ser	Val	Val
Ala	Val	Leu	Ala	Tyr	Val	Tyr	Ser	Gly	Arg	Val	Arg	Ser	Pro	Pro	Lys
Gly	Ala	Ser	Ala	Cys	Val	Asp	Asp	Asp	Cys	Cys	His	Val	Ala	Cys	Arg
Ser	Lys	Val	Asp	Phe	Met	Val	Glu	Val	Leu	Tyr	Leu	Ser	Phe	Val	Phe
Gln	Ile	Gln	Glu	Leu	Val	Thr	Leu	Tyr	Glu	Arg	Gln	Phe	Leu	Glu	Ile
Val	Asp	Lys	Val	Val	Val	Glu	Asp	Ile	Leu	Val	Ile	Phe	Lys	Leu	Asp
Thr	Leu	Cys	Gly	Thr	Thr	Tyr	Lys	Lys	Leu	Leu	Asp	Arg	Cys	Ile	Glu
Ile	Ile	Val	Lys	Ser	Asp	Ile	Glu	Leu	Val	Ser	Leu	Glu	Lys	Ser	Leu
Pro	Gln	His	Ile	Phe	Lys	Gln	Ile	Ile	Asp	Ile	Arg	Glu	Ala	Leu	Cys
Leu	Glu	Pro	Pro	Lys	Leu	Glu	Arg	His	Val	Lys	Asn	Ile	Tyr	Lys	Ala
Leu	Asp	Ser	Asp	Asp	Val	Glu	Leu	Val	Lys	Met	Leu	Leu	Leu	Glu	Gly
His	Thr	Asn	Leu	Asp	Glu	Ala	Tyr	Ala	Leu	His	Phe	Ala	Ile	Ala	His
Cys	Ala	Val	Lys	Thr	Ala	Tyr	Asp	Leu	Leu	Glu	Leu	Glu	Leu	Ala	Asp
Val	Asn	Leu	Arg	Asn	Pro	Arg	Gly	Tyr	Thr	Val	Leu	His	Val	Ala	Ala
Met	Arg	Lys	Glu	Pro	Lys	Leu	Ile	Ile	Ser	Leu	Leu	Met	Lys	Gly	Ala
Asn	Ile	Leu	Asp	Thr	Thr	Leu	Asp	Gly	Arg	Thr	Ala	Leu	Val	Ile	Val
Lys	Arg	Leu	Thr	Lys	Ala	Asp	Asp	Tyr	Lys	Thr	Ser	Thr	Glu	Asp	Gly
Thr	Pro	Ser	Leu	Lys	Gly	Gly	Leu	Cys	Ile	Glu	Val	Leu	Glu	His	Glu
Gln	Lys	Leu	Glu	Tyr	Leu	Ser	Pro	Ile	Glu	Ala	Ser	Leu	Ser	Leu	Pro
Val	Thr	Pro	Glu	Glu	Leu	Arg	Met	Arg	Leu	Leu	Tyr	Tyr	Glu	Asn	Arg
Val	Ala	Leu	Ala	Arg	Leu	Leu	Phe	Pro	Val	Glu	Thr	Glu	Thr	Val	Gln
Gly	Ile	Ala	Lys	Leu	Glu	Glu	Thr	Cys	Glu	Phe	Thr	Ala	Ser	Ser	Leu
Glu	Pro	Asp	His	His	Ile	Gly	Glu	Lys	Arg	Thr	Ser	Leu	Asp	Leu	Asn
Met	Ala	Pro	Phe	Gln	Ile	His	Glu	Lys	His	Leu	Ser	Arg	Leu	Arg	Ala

Leu Cys Lys Thr Val Glu Leu Gly Lys Arg Tyr Phe Lys Arg Cys Ser  
 500 505 510  
 Leu Asp His Phe Met Asp Thr Glu Asp Leu Asn His Leu Ala Ser Val  
 515 520 525  
 Glu Glu Asp Thr Pro Glu Lys Arg Leu Gln Lys Lys Gln Arg Tyr Met  
 530 535 540  
 Glu Leu Gln Glu Thr Leu Met Lys Thr Phe Ser Glu Asp Lys Glu Glu  
 545 550 555 560  
 Cys Gly Lys Ser Ser Thr Pro Lys Pro Thr Ser Ala Val Arg Ser Asn  
 565 570 575  
 Arg Lys Leu Ser His Arg Arg Leu Lys Val Asp Lys Arg Asp Phe Leu  
 580 585 590  
 Lys Arg Pro Tyr Gly Asn Gly Asp  
 595 600

<210> 21  
 <211> 28  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer NIM  
 1A

<400> 21  
 gakattattg tcaagtctaa tgtwgata 28

<210> 22  
 <211> 25  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer NIM  
 1B

<400> 22  
 aytkgaytck gatgatrttg artta 25

<210> 23  
 <211> 27  
 <212> DNA  
 <213> Artificial Sequence

<220>  
 <223> Description of Artificial Sequence: PCR primer NIM  
 1C

<400> 23  
 taaytcaaya tcatcmgart cmartgc 27

<210> 24  
 <211> 28  
 <212> DNA  
 <213> Artificial Sequence

<220>  
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1D

<220>  
<221> misc\_feature  
<222> (1)..(28)  
<223> n = a, t, c or g

<400> 24  
gttkagcmag nscaactcta ttttcaag 28

<210> 25  
<211> 32  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer NIM  
2A

<400> 25  
tgcatwgara twrttgtsaa gtctratgtw ga 32

<210> 26  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer NIM  
2B

<400> 26  
ggcaytggay tcw gatgatg ttgaryt 27

<210> 27  
<211> 27  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer NIM  
2C

<400> 27  
arytcaacat catcwgartc cartgcc 27

<210> 28  
<211> 31  
<212> DNA  
<213> Artificial Sequence

<220>  
<223> Description of Artificial Sequence: PCR primer NIM  
2D



<400> 28  
agttkagcma gdccaactck attttcaarr t 31

<210> 29  
<211> 659  
<212> DNA  
<213> Nicotiana tabacum

<220>  
<221> CDS  
<222> (1)..(657)  
<223> Tobacco A

<400> 29  
tgc atg gag att att gtc aag tct aat gtt gat atc ata acc ctt gat 48  
Cys Met Glu Ile Ile Val Lys Ser Asn Val Asp Ile Ile Thr Leu Asp  
1 5 10 15

aag gcc ttg cct cat gac att gta aaa caa att acc gat tca cga gca 96  
Lys Ala Leu Pro His Asp Ile Val Lys Gln Ile Thr Asp Ser Arg Ala  
20 25 30

gaa ctt ggt cta caa ggg cct gaa agc aat ggt ttt cct gat aaa cat 144  
Glu Leu Gly Leu Gln Gly Pro Glu Ser Asn Gly Phe Pro Asp Lys His  
35 40 45

gtt aag agg ata cat agg gca tta gat tct gat gat gtt gaa tta ctg 192  
Val Lys Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Leu  
50 55 60

cag atg ttg cta aga gag ggg cat act act cta gat gat gca tat gct 240  
Gln Met Leu Leu Arg Glu Gly His Thr Thr Leu Asp Asp Ala Tyr Ala  
65 70 75 80

ctc cac tat gct gta gca tat tgc gat gca aag act aca gca gaa ctt 288  
Leu His Tyr Ala Val Ala Tyr Cys Asp Ala Lys Thr Thr Ala Glu Leu  
85 90 95

cta gat ctt gca ctt gct gat gtt aat cat caa aat tca aga gga tac 336  
Leu Asp Leu Ala Leu Ala Asp Val Asn His Gln Asn Ser Arg Gly Tyr  
100 105 110

aca gtg ctg cat gtt gca gcc atg agg aaa gag cct aaa att ata gtg 384  
Thr Val Leu His Val Ala Ala Met Arg Lys Glu Pro Lys Ile Ile Val  
115 120 125

tcc ctt tta acc aaa gga gct aga cct tct gat ctg aca tcc gat ggc 432  
Ser Leu Leu Thr Lys Gly Ala Arg Pro Ser Asp Leu Thr Ser Asp Gly  
130 135 140

aga aaa gca ctt caa att gcc aag agg ctc act agg ctt gtg gat ttc 480  
Arg Lys Ala Leu Gln Ile Ala Lys Arg Leu Thr Arg Leu Val Asp Phe  
145 150 155 160

agt aag tct cca gag gaa gga aaa tct gct tcg aag gat cgg tta tgc 528  
Ser Lys Ser Pro Glu Glu Gly Lys Ser Ala Ser Lys Asp Arg Leu Cys  
165 170 175

att gag att ctg gag caa gca gaa aga aga gat cca ctg cta gga gaa 576  
Ile Glu Ile Leu Glu Gln Ala Glu Arg Arg Asp Pro Leu Leu Gly Glu

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                180                185                190
gct tct gta tct ctt gct atg gcg ggc gat gat ttg cgt atg aag ctg 624
Ala Ser Val Ser Leu Ala Met Ala Gly Asp Asp Leu Arg Met Lys Leu
                195                200                205

tta tac ctt gaa aat aga gtt ggc ctt gct caa ct 659
Leu Tyr Leu Glu Asn Arg Val Gly Leu Ala Gln
                210                215

<210> 30
<211> 219
<212> PRT
<213> Nicotiana tabacum

<400> 30
Cys Met Glu Ile Ile Val Lys Ser Asn Val Asp Ile Ile Thr Leu Asp
  1                    5                    10                    15
Lys Ala Leu Pro His Asp Ile Val Lys Gln Ile Thr Asp Ser Arg Ala
                20                25                30
Glu Leu Gly Leu Gln Gly Pro Glu Ser Asn Gly Phe Pro Asp Lys His
                35                40                45
Val Lys Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Leu
                50                55                60
Gln Met Leu Leu Arg Glu Gly His Thr Thr Leu Asp Asp Ala Tyr Ala
                65                70                75                80
Leu His Tyr Ala Val Ala Tyr Cys Asp Ala Lys Thr Thr Ala Glu Leu
                85                90                95
Leu Asp Leu Ala Leu Ala Asp Val Asn His Gln Asn Ser Arg Gly Tyr
                100                105                110
Thr Val Leu His Val Ala Ala Met Arg Lys Glu Pro Lys Ile Ile Val
                115                120                125
Ser Leu Leu Thr Lys Gly Ala Arg Pro Ser Asp Leu Thr Ser Asp Gly
                130                135                140
Arg Lys Ala Leu Gln Ile Ala Lys Arg Leu Thr Arg Leu Val Asp Phe
                145                150                155                160
Ser Lys Ser Pro Glu Glu Gly Lys Ser Ala Ser Lys Asp Arg Leu Cys
                165                170                175
Ile Glu Ile Leu Glu Gln Ala Glu Arg Arg Asp Pro Leu Leu Gly Glu
                180                185                190
Ala Ser Val Ser Leu Ala Met Ala Gly Asp Asp Leu Arg Met Lys Leu
                195                200                205
Leu Tyr Leu Glu Asn Arg Val Gly Leu Ala Gln
                210                215

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<210> 31  
 <211> 498  
 <212> DNA  
 <213> Nicotiana tabacum

<220>  
 <221> CDS  
 <222> (2)..(496)  
 <223> Tobacco B

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   Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Asn Glu  
     1                    5                    10                    15  
  
 tct gag ata agc tta gat gaa gcc tac gct ctt cat tat gct gtt gca 97  
 Ser Glu Ile Ser Leu Asp Glu Ala Tyr Ala Leu His Tyr Ala Val Ala  
           20                    25                    30  
  
 tat tgt gat ccc aag gtt gtg act gag gtt ctt gga ctg ggt gtt gct 145  
 Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu Gly Val Ala  
           35                    40                    45  
  
 gat gtc aat cta cgt aat act cgc ggt tac act gtg ctt cac att gct 193  
 Asp Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala  
           50                    55                    60  
  
 gcc atg cgt aag gag cca gca ata att gta tgc ctt ttg act aag gga 241  
 Ala Met Arg Lys Glu Pro Ala Ile Ile Val Ser Leu Leu Thr Lys Gly  
           65                    70                    75                    80  
  
 gct cat gtg tca gag att aca ttg gat ggg caa agt gct gtt agt atc 289  
 Ala His Val Ser Glu Ile Thr Leu Asp Gly Gln Ser Ala Val Ser Ile  
           85                    90                    95  
  
 tgt agg agg cta act agg cct aag gag tac cat gca aaa aca gaa caa 337  
 Cys Arg Arg Leu Thr Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln  
           100                    105                    110  
  
 ggc cag gaa gca aac aaa gat cgg gta tgt att gat gtt ttg gag aga 385  
 Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg  
           115                    120                    125  
  
 gag atg cgt cgc aac cca atg gct gga gat gca ttg ctt tct tcc caa 433  
 Glu Met Arg Arg Asn Pro Met Ala Gly Asp Ala Leu Leu Ser Ser Gln  
           130                    135                    140  
  
 atg ttg gcc gat gat ctg cac atg aaa ctg cac tat ttt gaa aat cga 481  
 Met Leu Ala Asp Asp Leu His Met Lys Leu His Tyr Phe Glu Asn Arg  
           145                    150                    155                    160  
  
 gtt gga ctt gct caa ct 498  
 Val Gly Leu Ala Gln  
                     165

<210> 32  
 <211> 165  
 <212> PRT  
 <213> Nicotiana tabacum

&lt;400&gt; 32

Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Asn Glu  
 1 5 10 15

Ser Glu Ile Ser Leu Asp Glu Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30

Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu Gly Val Ala  
 35 40 45

Asp Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala  
 50 55 60

Ala Met Arg Lys Glu Pro Ala Ile Ile Val Ser Leu Leu Thr Lys Gly  
 65 70 75 80

Ala His Val Ser Glu Ile Thr Leu Asp Gly Gln Ser Ala Val Ser Ile  
 85 90 95

Cys Arg Arg Leu Thr Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln  
 100 105 110

Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg  
 115 120 125

Glu Met Arg Arg Asn Pro Met Ala Gly Asp Ala Leu Leu Ser Ser Gln  
 130 135 140

Met Leu Ala Asp Asp Leu His Met Lys Leu His Tyr Phe Glu Asn Arg  
 145 150 155 160

Val Gly Leu Ala Gln  
 165

&lt;210&gt; 33

&lt;211&gt; 498

&lt;212&gt; DNA

&lt;213&gt; Nicotiana tabacum

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (2)..(496)

&lt;223&gt; Tobacco C

&lt;400&gt; 33

g gca ctg gac tcw gat gat gtt gag ttt gtc aag ctt cta ctg agt gag 49  
 Ala Leu Asp Xaa Asp Asp Val Glu Phe Val Lys Leu Leu Leu Ser Glu  
 1 5 10 15

tct aac ata agc tta gat gaa gcc tac gct ctt cat tat gct gtg gca 97  
 Ser Asn Ile Ser Leu Asp Glu Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30

tat tgt gat ccc aag gtt gtg act gag gtt ctt gga ctg ggt gtt gcg 145  
 Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu Gly Val Ala  
 35 40 45

gat gtc aac cta cgt aat act cgt ggt tac act gtg ctt cac att gct 193  
 Asp Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala

50	55	60	
tcc atg cgt aag gag cca gca gta att gta tgc ctt ttg act aag gga			241
Ser Met Arg Lys Glu Pro Ala Val Ile Val Ser Leu Leu Thr Lys Gly			
65	70	75	80
gct cgt gca tca gag act aca ttg gat ggg cag agt gct gtt agt atc			289
Ala Arg Ala Ser Glu Thr Thr Leu Asp Gly Gln Ser Ala Val Ser Ile			
85	90		95
tgt agg agg ctg act agg cct aag gag tac cat gca aaa aca gaa caa			337
Cys Arg Arg Leu Thr Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln			
100	105		110
ggc cag gaa gca aac aaa gat cgg gta tgt att gat gtt ttg gag aga			385
Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg			
115	120		125
gag atg cgt cgc aac cca atg gct gga gat gca ttg ttt tct tcc cca			433
Glu Met Arg Arg Asn Pro Met Ala Gly Asp Ala Leu Phe Ser Ser Pro			
130	135		140
atg ttg gcc gat gat ctg cac atg aaa ctg cac tac ctt gaa aat aga			481
Met Leu Ala Asp Asp Leu His Met Lys Leu His Tyr Leu Glu Asn Arg			
145	150	155	160
gtt ggc ctg gct caa ct			498
Val Gly Leu Ala Gln			
165			
<210> 34			
<211> 165			
<212> PRT			
<213> Nicotiana tabacum			
<400> 34			
Ala Leu Asp Xaa Asp Asp Val Glu Phe Val Lys Leu Leu Leu Ser Glu			
1	5	10	15
Ser Asn Ile Ser Leu Asp Glu Ala Tyr Ala Leu His Tyr Ala Val Ala			
20	25	30	
Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu Gly Val Ala			
35	40	45	
Asp Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala			
50	55	60	
Ser Met Arg Lys Glu Pro Ala Val Ile Val Ser Leu Leu Thr Lys Gly			
65	70	75	80
Ala Arg Ala Ser Glu Thr Thr Leu Asp Gly Gln Ser Ala Val Ser Ile			
85	90		95
Cys Arg Arg Leu Thr Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln			
100	105		110
Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg			
115	120		125

Glu Met Arg Arg Asn Pro Met Ala Gly Asp Ala Leu Phe Ser Ser Pro  
 130 135 140

Met Leu Ala Asp Asp Leu His Met Lys Leu His Tyr Leu Glu Asn Arg  
 145 150 155 160

Val Gly Leu Ala Gln  
 165

<210> 35  
 <211> 399  
 <212> DNA  
 <213> Nicotiana tabacum

<220>  
 <221> CDS  
 <222> (1)..(399)  
 <223> Tobacco D

<400> 35  
 act gat tcg gat gat gtt gag tta ctt aag tta ctt ctt gaa gag tct 48  
 Thr Asp Ser Asp Asp Val Glu Leu Leu Lys Leu Leu Leu Glu Glu Ser  
 1 5 10 15

aat gtc act tta gac gat gct tgt gct ctt cat tat gca gct gct tat 96  
 Asn Val Thr Leu Asp Asp Ala Cys Ala Leu His Tyr Ala Ala Ala Tyr  
 20 25 30

tgt aac tcc aag gtt gtg aat gag gtc ctc gag ctg gat tta gct gat 144  
 Cys Asn Ser Lys Val Val Asn Glu Val Leu Glu Leu Asp Leu Ala Asp  
 35 40 45

gtc aat ctt cag aac tcc cga gga tat aac gtc ctt cac gtt gct gct 192  
 Val Asn Leu Gln Asn Ser Arg Gly Tyr Asn Val Leu His Val Ala Ala  
 50 55 60

aga aga aag gag cca tca ata ata atg gga cta ctt gaa aaa gga gca 240  
 Arg Arg Lys Glu Pro Ser Ile Ile Met Gly Leu Leu Glu Lys Gly Ala  
 65 70 75 80

tct ttc ttg aat act aca cgg gat gga aac aca gca cta tct atc tgt 288  
 Ser Phe Leu Asn Thr Thr Arg Asp Gly Asn Thr Ala Leu Ser Ile Cys  
 85 90 95

cgg aga ttg act cgg cca aag gat tat aat gag cca aca aag caa ggg 336  
 Arg Arg Leu Thr Arg Pro Lys Asp Tyr Asn Glu Pro Thr Lys Gln Gly  
 100 105 110

aaa gaa act aat aag gac cgc ata tgc att gat att ttg gag aga gag 384  
 Lys Glu Thr Asn Lys Asp Arg Ile Cys Ile Asp Ile Leu Glu Arg Glu  
 115 120 125

acg aat agg aat cct 399  
 Thr Asn Arg Asn Pro  
 130

<210> 36  
 <211> 133

&lt;212&gt; PRT

&lt;213&gt; Nicotiana tabacum

&lt;400&gt; 36

Thr Asp Ser Asp Asp Val Glu Leu Leu Lys Leu Leu Leu Glu Glu Ser  
 1 5 10 15

Asn Val Thr Leu Asp Asp Ala Cys Ala Leu His Tyr Ala Ala Ala Tyr  
 20 25 30

Cys Asn Ser Lys Val Val Asn Glu Val Leu Glu Leu Asp Leu Ala Asp  
 35 40 45

Val Asn Leu Gln Asn Ser Arg Gly Tyr Asn Val Leu His Val Ala Ala  
 50 55 60

Arg Arg Lys Glu Pro Ser Ile Ile Met Gly Leu Leu Glu Lys Gly Ala  
 65 70 75 80

Ser Phe Leu Asn Thr Thr Arg Asp Gly Asn Thr Ala Leu Ser Ile Cys  
 85 90 95

Arg Arg Leu Thr Arg Pro Lys Asp Tyr Asn Glu Pro Thr Lys Gln Gly  
 100 105 110

Lys Glu Thr Asn Lys Asp Arg Ile Cys Ile Asp Ile Leu Glu Arg Glu  
 115 120 125

Thr Asn Arg Asn Pro  
 130

&lt;210&gt; 37

&lt;211&gt; 498

&lt;212&gt; DNA

&lt;213&gt; Lycopersicon esculentum

&lt;220&gt;

&lt;221&gt; CDS

&lt;222&gt; (2)..(496)

&lt;223&gt; Tomato A

&lt;400&gt; 37

g gca ttg gat tct gat gat gtt gag tta cta agg atg ttg ctt aaa gag 49  
 Ala Leu Asp Ser Asp Asp Val Glu Leu Leu Arg Met Leu Leu Lys Glu  
 1 5 10 15

ggg cat act act ctt gat gat gca tat gct ctc cac tat gct gta gca 97  
 Gly His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30

tat tgc gat gca aag act aca gca gaa ctt tta gat ctt tca ctt gct 145  
 Tyr Cys Asp Ala Lys Thr Thr Ala Glu Leu Leu Asp Leu Ser Leu Ala  
 35 40 45

gat gtt aat cat caa aat cct aga gga cac acg gta ctt cat gtt gct 193  
 Asp Val Asn His Gln Asn Pro Arg Gly His Thr Val Leu His Val Ala  
 50 55 60

gcc atg agg aaa gaa cct aaa att ata gtg tcc ctt tta acc aaa gga 241

Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu Leu Thr Lys Gly  
65 70 75 80

gct aga cct tct gat ctg aca tcc gat ggc aaa aaa gca ctt caa att 289  
Ala Arg Pro Ser Asp Leu Thr Ser Asp Gly Lys Lys Ala Leu Gln Ile  
85 90 95

gct aag agg ctc act agg ctt gta gat ttt acc aag tct aca gag gaa 337  
Ala Lys Arg Leu Thr Arg Leu Val Asp Phe Thr Lys Ser Thr Glu Glu  
100 105 110

gga aaa tct gct cca aag gat cgg tta tgc att gag att ctg gag caa 385  
Gly Lys Ser Ala Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu Glu Gln  
115 120 125

gca gaa aga aga gat cca cta cta gga gaa gct tca tta tct ctt gct 433  
Ala Glu Arg Arg Asp Pro Leu Leu Gly Glu Ala Ser Leu Ser Leu Ala  
130 135 140

atg gca ggc gat gat ttg cgt atg aag ctg tta tac ctt gaa aat aga 481  
Met Ala Gly Asp Asp Leu Arg Met Lys Leu Tyr Leu Glu Asn Arg  
145 150 155 160

gtt ggc ctt gct aaa ct 498  
Val Gly Leu Ala Lys  
165

<210> 38  
<211> 165  
<212> PRT  
<213> Lycopersicon esculentum

<400> 38  
Ala Leu Asp Ser Asp Asp Val Glu Leu Leu Arg Met Leu Leu Lys Glu  
1 5 10 15

Gly His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His Tyr Ala Val Ala  
20 25 30

Tyr Cys Asp Ala Lys Thr Thr Ala Glu Leu Leu Asp Leu Ser Leu Ala  
35 40 45

Asp Val Asn His Gln Asn Pro Arg Gly His Thr Val Leu His Val Ala  
50 55 60

Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu Leu Thr Lys Gly  
65 70 75 80

Ala Arg Pro Ser Asp Leu Thr Ser Asp Gly Lys Lys Ala Leu Gln Ile  
85 90 95

Ala Lys Arg Leu Thr Arg Leu Val Asp Phe Thr Lys Ser Thr Glu Glu  
100 105 110

Gly Lys Ser Ala Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu Glu Gln  
115 120 125

Ala Glu Arg Arg Asp Pro Leu Leu Gly Glu Ala Ser Leu Ser Leu Ala  
130 135 140



Met Ala Gly Asp Asp Leu Arg Met Lys Leu Leu Tyr Leu Glu Asn Arg  
 145 150 155 160

Val Gly Leu Ala Lys  
 165

<210> 39  
 <211> 498  
 <212> DNA  
 <213> Beta vulgaris

<220>  
 <221> CDS  
 <222> (2)..(496)  
 <223> Sugarbeet

<400> 39  
 g gca ttg gat tct gat gat gtt gag tta gtc aga atg ctt tta aaa gag 49  
 Ala Leu Asp Ser Asp Val Glu Leu Val Arg Met Leu Leu Lys Glu  
 1 5 10 15

cgc cat aca act cta gat gat gca tat gcc ctt cac tat gct gtg gca 97  
 Arg His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30

cat tgt gat gcc aag acc acc acg gag ctt ctt gag ctt ggg ctt gca 145  
 His Cys Asp Ala Lys Thr Thr Thr Glu Leu Leu Glu Leu Gly Leu Ala  
 35 40 45

gat gtt aat ctt aga aat cta agg ggt cac act gtg cta cat gtg gca 193  
 Asp Val Asn Leu Arg Asn Leu Arg Gly His Thr Val Leu His Val Ala  
 50 55 60

gcc atg aga aaa gag cct aag ata att gta tcc ttg tta acc aag gga 241  
 Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu Leu Thr Lys Gly  
 65 70 75 80

gcc cat ccg tct gat ata aca tca gat gat aaa aaa gca ctg cag ata 289  
 Ala His Pro Ser Asp Ile Thr Ser Asp Lys Lys Ala Leu Gln Ile  
 85 90 95

gca aag aga cta aca aaa gct gtg gac ttc tat aaa act aca gaa caa 337  
 Ala Lys Arg Leu Thr Lys Ala Val Asp Phe Tyr Lys Thr Thr Glu Gln  
 100 105 110

gga aaa gat gca cca aag gat cgg ttg tgc att gaa ata ctg gag caa 385  
 Gly Lys Asp Ala Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu Glu Gln  
 115 120 125

gct gaa aga aga gaa cca ttg cta gga gaa ggt tct gtt tct ctt gca 433  
 Ala Glu Arg Arg Glu Pro Leu Leu Gly Glu Gly Ser Val Ser Leu Ala  
 130 135 140

aag gca gga gat gat ctg cgt atg aag cta tta tac ctt gaa aat cga 481  
 Lys Ala Gly Asp Asp Leu Arg Met Lys Leu Leu Tyr Leu Glu Asn Arg  
 145 150 155 160

gtt ggc ctt gct caa ct 498  
 Val Gly Leu Ala Gln

165

<210> 40  
 <211> 165  
 <212> PRT  
 <213> Beta vulgaris

<400> 40  
 Ala Leu Asp Ser Asp Val Glu Leu Val Arg Met Leu Leu Lys Glu  
     1                    5                    10                    15  
 Arg His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His Tyr Ala Val Ala  
                     20                    25                    30  
 His Cys Asp Ala Lys Thr Thr Thr Glu Leu Leu Glu Leu Gly Leu Ala  
                     35                    40                    45  
 Asp Val Asn Leu Arg Asn Leu Arg Gly His Thr Val Leu His Val Ala  
                     50                    55                    60  
 Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu Leu Thr Lys Gly  
     65                    70                    75                    80  
 Ala His Pro Ser Asp Ile Thr Ser Asp Asp Lys Lys Ala Leu Gln Ile  
                     85                    90                    95  
 Ala Lys Arg Leu Thr Lys Ala Val Asp Phe Tyr Lys Thr Thr Glu Gln  
                     100                    105                    110  
 Gly Lys Asp Ala Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu Glu Gln  
                     115                    120                    125  
 Ala Glu Arg Arg Glu Pro Leu Leu Gly Glu Gly Ser Val Ser Leu Ala  
     130                    135                    140  
 Lys Ala Gly Asp Asp Leu Arg Met Lys Leu Leu Tyr Leu Glu Asn Arg  
     145                    150                    155                    160  
 Val Gly Leu Ala Gln  
                     165

<210> 41  
 <211> 498  
 <212> DNA  
 <213> Helianthus annuus

<220>  
 <221> CDS  
 <222> (2)..(496)  
 <223> Sunflower A

<400> 41  
 g gca ttg gat tct gat gat gtt gag yta gtc aca atg tta tta cga gaa 49  
   Ala Leu Asp Ser Asp Asp Val Glu Xaa Val Thr Met Leu Leu Arg Glu  
     1                    5                    10                    15  
 ggt cat act tca tta gac ggt tct tgc gct ctt cat tac gct gtt gcg 97  
 Gly His Thr Ser Leu Asp Gly Ser Cys Ala Leu His Tyr Ala Val Ala

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                20                25                30
tac gca gat gct aaa acg aca acc gaa tta ctg gat tta gca ctt gct 145
Tyr Ala Asp Ala Lys Thr Thr Thr Glu Leu Leu Asp Leu Ala Leu Ala
      35                40                45

gac gta aat cat aaa aac tcg agg ggt ttt acc gta ctt cat gtt gcc 193
Asp Val Asn His Lys Asn Ser Arg Gly Phe Thr Val Leu His Val Ala
      50                55                60

gct atg aga aaa gag cgg agt att atc gtt tcg ctt ctt acg aaa ggg 241
Ala Met Arg Lys Glu Pro Ser Ile Ile Val Ser Leu Leu Thr Lys Gly
      65                70                75                80

gcc cga ccc tcg gat ctc acc cct gat ggg aga aaa gca cta cag att 289
Ala Arg Pro Ser Asp Leu Thr Pro Asp Gly Arg Lys Ala Leu Gln Ile
      85                90                95

tcg aag agg ttg acc aga gcg gtt gac tat tac aag tca aac gag gat 337
Ser Lys Arg Leu Thr Arg Ala Val Asp Tyr Tyr Lys Ser Asn Glu Asp
      100                105                110

gat aaa gag tca acg aaa ggt cgt ttg tgt att gag ata ttg gaa caa 385
Asp Lys Glu Ser Thr Lys Gly Arg Leu Cys Ile Glu Ile Leu Glu Gln
      115                120                125

gcc gaa aga aga aat cca ttg tta ggt gaa gct tcg gct tct ctt gca 433
Ala Glu Arg Arg Asn Pro Leu Leu Gly Glu Ala Ser Ala Ser Leu Ala
      130                135                140

atg gcc gga gat gat ttg cgt gga aag ttg ttg tac ctt gaa aat cga 481
Met Ala Gly Asp Asp Leu Arg Gly Lys Leu Leu Tyr Leu Glu Asn Arg
      145                150                155                160

gtt ggc ctg gct caa ct 498
Val Gly Leu Ala Gln
      165

<210> 42
<211> 165
<212> PRT
<213> Helianthus annuus

<400> 42
Ala Leu Asp Ser Asp Asp Val Glu Xaa Val Thr Met Leu Leu Arg Glu
  1                5                10                15

Gly His Thr Ser Leu Asp Gly Ser Cys Ala Leu His Tyr Ala Val Ala
      20                25                30

Tyr Ala Asp Ala Lys Thr Thr Thr Glu Leu Leu Asp Leu Ala Leu Ala
      35                40                45

Asp Val Asn His Lys Asn Ser Arg Gly Phe Thr Val Leu His Val Ala
      50                55                60

Ala Met Arg Lys Glu Pro Ser Ile Ile Val Ser Leu Leu Thr Lys Gly
      65                70                75                80

Ala Arg Pro Ser Asp Leu Thr Pro Asp Gly Arg Lys Ala Leu Gln Ile

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	85		90		95
Ser Lys Arg Leu Thr Arg Ala Val Asp Tyr Tyr Lys Ser Asn Glu Asp					
	100		105		110
Asp Lys Glu Ser Thr Lys Gly Arg Leu Cys Ile Glu Ile Leu Glu Gln					
	115		120		125
Ala Glu Arg Arg Asn Pro Leu Leu Gly Glu Ala Ser Ala Ser Leu Ala					
	130		135		140
Met Ala Gly Asp Asp Leu Arg Gly Lys Leu Leu Tyr Leu Glu Asn Arg					
	145		150		155
Val Gly Leu Ala Gln					160
					165

<210> 43  
 <211> 498  
 <212> DNA  
 <213> Helianthus annuus

<220>  
 <221> CDS  
 <222> (2)..(496)  
 <223> Sunflower B

<400> 43  
 g gca ttg gac tct gat gat gtt gag ctt gtg aaa atg att tta gac gaa 49  
 Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Met Ile Leu Asp Glu  
 1 5 10 15

tcc aaa atc acg tta gat gaa gcc tgc gct ctt cat tat gcg gtc atg 97  
 Ser Lys Ile Thr Leu Asp Glu Ala Cys Ala Leu His Tyr Ala Val Met  
 20 25 30

tat tgt aat caa gaa gtt gct aag gag att ctt aac tta aac cgt gcg 145  
 Tyr Cys Asn Gln Glu Val Ala Lys Glu Ile Leu Asn Leu Asn Arg Ala  
 35 40 45

gat gtt aat ctt aga aac tca cga gat tac acc gtg ctt cat gtt gct 193  
 Asp Val Asn Leu Arg Asn Ser Arg Asp Tyr Thr Val Leu His Val Ala  
 50 55 60

gcc atg cgt aaa gaa cca tca ctt att gtt tcg att cta agc aaa ggc 241  
 Ala Met Arg Lys Glu Pro Ser Leu Ile Val Ser Ile Leu Ser Lys Gly  
 65 70 75 80

gcg tgt gca tcg gat act act ttt gat gga caa agt gcg gtt agt att 289  
 Ala Cys Ala Ser Asp Thr Thr Phe Asp Gly Gln Ser Ala Val Ser Ile  
 85 90 95

tgc agg aga cga aca agg ccc aag gat tat tat gtg aaa acc gaa cac 337  
 Cys Arg Arg Arg Thr Arg Pro Lys Asp Tyr Tyr Val Lys Thr Glu His  
 100 105 110

ggg caa gaa aca aat aaa gat cgt ata tgc atc gat gtt ttg gag cgg 385  
 Gly Gln Glu Thr Asn Lys Asp Arg Ile Cys Ile Asp Val Leu Glu Arg  
 115 120 125

gaa ata aag agg aat ccg atg ata ggc gat gtt tcc gtg tgt tct tca 433  
 Glu Ile Lys Arg Asn Pro Met Ile Gly Asp Val Ser Val Cys Ser Ser  
 130 135 140

gca gtg gct gat gat ttg cat atg aat tta ctc tac ttt gaa aat cga 481  
 Ala Val Ala Asp Asp Leu His Met Asn Leu Leu Tyr Phe Glu Asn Arg  
 145 150 155 160

gtt ggc ctt gct caa ct 498  
 Val Gly Leu Ala Gln  
 165

<210> 44  
 <211> 165  
 <212> PRT  
 <213> Helianthus annuus

<400> 44  
 Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Met Ile Leu Asp Glu  
 1 5 10 15  
 Ser Lys Ile Thr Leu Asp Glu Ala Cys Ala Leu His Tyr Ala Val Met  
 20 25 30  
 Tyr Cys Asn Gln Glu Val Ala Lys Glu Ile Leu Asn Leu Asn Arg Ala  
 35 40 45  
 Asp Val Asn Leu Arg Asn Ser Arg Asp Tyr Thr Val Leu His Val Ala  
 50 55 60  
 Ala Met Arg Lys Glu Pro Ser Leu Ile Val Ser Ile Leu Ser Lys Gly  
 65 70 75 80  
 Ala Cys Ala Ser Asp Thr Thr Phe Asp Gly Gln Ser Ala Val Ser Ile  
 85 90 95  
 Cys Arg Arg Arg Thr Arg Pro Lys Asp Tyr Tyr Val Lys Thr Glu His  
 100 105 110  
 Gly Gln Glu Thr Asn Lys Asp Arg Ile Cys Ile Asp Val Leu Glu Arg  
 115 120 125  
 Glu Ile Lys Arg Asn Pro Met Ile Gly Asp Val Ser Val Cys Ser Ser  
 130 135 140  
 Ala Val Ala Asp Asp Leu His Met Asn Leu Leu Tyr Phe Glu Asn Arg  
 145 150 155 160  
 Val Gly Leu Ala Gln  
 165

<210> 45  
 <211> 653  
 <212> DNA  
 <213> Solanum tuberosum  
 <220>

&lt;221&gt; CDS

&lt;222&gt; (1)..(651)

&lt;223&gt; Potato A

&lt;400&gt; 45

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gak att att gtc aag tct aat gtt gat atc ata acc ctt gat aag tcc 48
Xaa Ile Ile Val Lys Ser Asn Val Asp Ile Ile Thr Leu Asp Lys Ser
  1           5           10          15

ttg cct cat gac atc gta aaa caa atc act gat tca cgt gct gaa ctt 96
Leu Pro His Asp Ile Val Lys Gln Ile Thr Asp Ser Arg Ala Glu Leu
          20          25          30

ggg cta caa ggg cct gaa agc aat ggt ttt cct gat aaa cat gtt aag 144
Gly Leu Gln Gly Pro Glu Ser Asn Gly Phe Pro Asp Lys His Val Lys
          35          40          45

agg ata cat agg gca ttg gac tct gat gat gtt gag tta cta agg atg 192
Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Leu Arg Met
          50          55          60

ttg ctt aaa gaa ggg cat act act ctc gat gat gca tat gct ctc cac 240
Leu Leu Lys Glu Gly His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His
          65          70          75          80

tat gct gta gca tat tgc gat gca aag act aca gca gaa ctt tta gat 288
Tyr Ala Val Ala Tyr Cys Asp Ala Lys Thr Thr Ala Glu Leu Leu Asp
          85          90          95

ctt tca ctt gct gat gtt aat cat caa aat cct aga gga tac acg gta 336
Leu Ser Leu Ala Asp Val Asn His Gln Asn Pro Arg Gly Tyr Thr Val
          100          105          110

ctt cat gtt gct gcc atg agg aaa gag cct aaa att ata gtg tcc ctt 384
Leu His Val Ala Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu
          115          120          125

tta acc aaa gga gct aga cct tct gat ctg aca tct gat ggc aaa aaa 432
Leu Thr Lys Gly Ala Arg Pro Ser Asp Leu Thr Ser Asp Gly Lys Lys
          130          135          140

gca ctt caa att gct aag agg ctc act agg ctt gtg gat ttt act aag 480
Ala Leu Gln Ile Ala Lys Arg Leu Thr Arg Leu Val Asp Phe Thr Lys
          145          150          155          160

tct aca gag gaa gga aaa tct gct cca aaa gat cgg tta tgc att gag 528
Ser Thr Glu Glu Gly Lys Ser Ala Pro Lys Asp Arg Leu Cys Ile Glu
          165          170          175

att ctg gag caa gca gaa aga aga gat cca cta cta gga gaa gct tca 576
Ile Leu Glu Gln Ala Glu Arg Arg Asp Pro Leu Leu Gly Glu Ala Ser
          180          185          190

tta tct ctt gct atg gca ggc gat gat ttg cgt atg aag ctg tta tac 624
Leu Ser Leu Ala Met Ala Gly Asp Asp Leu Arg Met Lys Leu Leu Tyr
          195          200          205

ctt gaa aat cga gtt ggc ctk gct caa ct 653
Leu Glu Asn Arg Val Gly Xaa Ala Gln
          210          215

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<210> 46  
 <211> 217  
 <212> PRT  
 <213> Solanum tuberosum

<400> 46  
 Xaa Ile Ile Val Lys Ser Asn Val Asp Ile Ile Thr Leu Asp Lys Ser  
     1                    5                    10                    15  
 Leu Pro His Asp Ile Val Lys Gln Ile Thr Asp Ser Arg Ala Glu Leu  
                     20                    25                    30  
 Gly Leu Gln Gly Pro Glu Ser Asn Gly Phe Pro Asp Lys His Val Lys  
                     35                    40                    45  
 Arg Ile His Arg Ala Leu Asp Ser Asp Asp Val Glu Leu Leu Arg Met  
                     50                    55                    60  
 Leu Leu Lys Glu Gly His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His  
                     65                    70                    75                    80  
 Tyr Ala Val Ala Tyr Cys Asp Ala Lys Thr Thr Ala Glu Leu Leu Asp  
                     85                    90                    95  
 Leu Ser Leu Ala Asp Val Asn His Gln Asn Pro Arg Gly Tyr Thr Val  
                     100                    105                    110  
 Leu His Val Ala Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu  
                     115                    120                    125  
 Leu Thr Lys Gly Ala Arg Pro Ser Asp Leu Thr Ser Asp Gly Lys Lys  
                     130                    135                    140  
 Ala Leu Gln Ile Ala Lys Arg Leu Thr Arg Leu Val Asp Phe Thr Lys  
                     145                    150                    155                    160  
 Ser Thr Glu Glu Gly Lys Ser Ala Pro Lys Asp Arg Leu Cys Ile Glu  
                     165                    170                    175  
 Ile Leu Glu Gln Ala Glu Arg Arg Asp Pro Leu Leu Gly Glu Ala Ser  
                     180                    185                    190  
 Leu Ser Leu Ala Met Ala Gly Asp Asp Leu Arg Met Lys Leu Leu Tyr  
                     195                    200                    205  
 Leu Glu Asn Arg Val Gly Xaa Ala Gln  
                     210                    215

<210> 47  
 <211> 498  
 <212> DNA  
 <213> Solanum tuberosum

<220>  
 <221> CDS  
 <222> (2)..(496)  
 <223> Potato B

&lt;400&gt; 47

g gca ttg gat tca gat gat gtt gag ttt gtc aag ctt cta ctt aat gag 49  
 Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Asn Glu  
 1 5 10 15  
  
 tct gac ata agt tta gat gga gcc tac gct ctt cat tac gct gtt gca 97  
 Ser Asp Ile Ser Leu Asp Gly Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30  
  
 tat tgt gac ccc aag gtt gtt act gag gtt ctt gga ctg ggt gtt gct 145  
 Tyr Cys Asp Pro Lys Val Val Thr Arg Glu Val Leu Gly Leu Gly Val Ala  
 35 40 45  
  
 aat gtc aac ctt cgg aat aca cgt ggt tac act gtg ctt cac att gct 193  
 Asn Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala  
 50 55 60  
  
 gcc atg cgt aag gaa ccc tca atc att gta tca ctt ttg act aag gga 241  
 Ala Met Arg Lys Glu Pro Ser Ile Ile Val Ser Leu Leu Thr Lys Gly  
 65 70 75 80  
  
 gct cat gca tca gaa att aca ttg gat ggg cag agt gct gtt ggc atc 289  
 Ala His Ala Ser Glu Ile Thr Leu Asp Gly Gln Ser Ala Val Gly Ile  
 85 90 95  
  
 tgt agg agg ctg agt agg cct aag gag tac cat gca aaa aca gaa caa 337  
 Cys Arg Arg Leu Ser Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln  
 100 105 110  
  
 ggc cag gaa gca aac aaa gat cgg gta tgt att gat gtt ttg gag aga 385  
 Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg  
 115 120 125  
  
 gag atg cgt cac aac cca atg acc gga gat gca tta ttt tct tcc ccc 433  
 Glu Met Arg His Asn Pro Met Thr Gly Asp Ala Leu Phe Ser Ser Pro  
 130 135 140  
  
 atg ttg gcc gat gat ctg ccc atg aaa ctg ctc tac ctt gaa aat cga 481  
 Met Leu Ala Asp Asp Leu Pro Met Lys Leu Leu Tyr Leu Glu Asn Arg  
 145 150 155 160  
  
 gtt ggc ctt gct aaa ct 498  
 Val Gly Leu Ala Lys  
 165

&lt;210&gt; 48

&lt;211&gt; 165

&lt;212&gt; PRT

&lt;213&gt; Solanum tuberosum

&lt;400&gt; 48

Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Asn Glu  
 1 5 10 15  
  
 Ser Asp Ile Ser Leu Asp Gly Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30  
  
 Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu Gly Val Ala  
 35 40 45



Asn Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala  
 50 55 60  
 Ala Met Arg Lys Glu Pro Ser Ile Ile Val Ser Leu Leu Thr Lys Gly  
 65 70 75 80  
 Ala His Ala Ser Glu Ile Thr Leu Asp Gly Gln Ser Ala Val Gly Ile  
 85 90 95  
 Cys Arg Arg Leu Ser Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln  
 100 105 110  
 Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg  
 115 120 125  
 Glu Met Arg His Asn Pro Met Thr Gly Asp Ala Leu Phe Ser Ser Pro  
 130 135 140  
 Met Leu Ala Asp Asp Leu Pro Met Lys Leu Leu Tyr Leu Glu Asn Arg  
 145 150 155 160  
 Val Gly Leu Ala Lys  
 165

<210> 49  
 <211> 477  
 <212> DNA  
 <213> Solanum tuberosum

<220>  
 <221> CDS  
 <222> (2)..(475)  
 <223> Potato C

<400> 49  
 g gca ctg gac tct gat gat gtt gag ttt gtc aag ctt cta ctt aat gag 49  
 Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Asn Glu  
 1 5 10 15  
 tct gac ata agt tta gat gga gcc tac gct ctt cat tac gct gtt gca 97  
 Ser Asp Ile Ser Leu Asp Gly Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30  
 tat tgt gac ccc aag gtt gtt act gag gtt ctt gga ctg ggt gtt gct 145  
 Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu Gly Val Ala  
 35 40 45  
 aat gtc aac ctt cgg aat aca cgt ggt tac act gtg ctt cac att gct 193  
 Asn Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala  
 50 55 60  
 gcc atg cgt aag gaa ccc tca atc att gta tca ctt ttg act aag gga 241  
 Ala Met Arg Lys Glu Pro Ser Ile Ile Val Ser Leu Leu Thr Lys Gly  
 65 70 75 80  
 gct cat gca tca gaa att aca ttg gat ggg cag agt gct gtt agc atc 289  
 Ala His Ala Ser Glu Ile Thr Leu Asp Gly Gln Ser Ala Val Ser Ile  
 85 90 95

tgt agg agg ctg act agg cct aag gag tac cat gca aaa aca gaa caa 337  
 Cys Arg Arg Leu Thr Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln  
                   100                  105                  110

ggc cag gaa gca aac aaa gat cgg gta tgt att gat gtt ttg gag aga 385  
 Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg  
                   115                  120                  125

gag atg cgt cgc aac cca atg acc gga gat gca tta ttt tct tcc ccc 433  
 Glu Met Arg Arg Asn Pro Met Thr Gly Asp Ala Leu Phe Ser Ser Pro  
                   130                  135                  140

atg aaa cag ctg tac ctt gaa aat aga gtt ggc ctt gct aaa ct 477  
 Met Lys Gln Leu Tyr Leu Glu Asn Arg Val Gly Leu Ala Lys  
                   145                  150                  155

<210> 50  
 <211> 158  
 <212> PRT  
 <213> Solanum tuberosum

<400> 50  
 Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Asn Glu  
   1                  5                  10                  15  
 Ser Asp Ile Ser Leu Asp Gly Ala Tyr Ala Leu His Tyr Ala Val Ala  
                   20                  25                  30  
 Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu Gly Val Ala  
                   35                  40                  45  
 Asn Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu His Ile Ala  
                   50                  55                  60  
 Ala Met Arg Lys Glu Pro Ser Ile Ile Val Ser Leu Leu Thr Lys Gly  
                   65                  70                  75                  80  
 Ala His Ala Ser Glu Ile Thr Leu Asp Gly Gln Ser Ala Val Ser Ile  
                   85                  90                  95  
 Cys Arg Arg Leu Thr Arg Pro Lys Glu Tyr His Ala Lys Thr Glu Gln  
                   100                  105                  110  
 Gly Gln Glu Ala Asn Lys Asp Arg Val Cys Ile Asp Val Leu Glu Arg  
                   115                  120                  125  
 Glu Met Arg Arg Asn Pro Met Thr Gly Asp Ala Leu Phe Ser Ser Pro  
                   130                  135                  140  
 Met Lys Gln Leu Tyr Leu Glu Asn Arg Val Gly Leu Ala Lys  
                   145                  150                  155

<210> 51  
 <211> 501  
 <212> DNA  
 <213> Brassica napus

<220>

&lt;221&gt; CDS

&lt;222&gt; (2) .. (499)

&lt;223&gt; Canola A

&lt;400&gt; 51

g gca ttg gat tct gat gat gtt gag ttt gtg aag ttg ctt ttg act gag 49

Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Thr Glu  
1 5 10 15

tca gat atc act cta gat gaa gcc aat ggt ctt cat tac tca gtg gtg 97

Ser Asp Ile Thr Leu Asp Glu Ala Asn Gly Leu His Tyr Ser Val Val  
20 25 30

tat agt gat ccc aaa gtt gtt gcc gag att ctt act ctt gat atg ggt 145

Tyr Ser Asp Pro Lys Val Val Ala Glu Ile Leu Thr Leu Asp Met Gly  
35 40 45

gat gtc aac cac aga aac tca cgt ggc tac acg gtt ctt cat ctc gca 193

Asp Val Asn His Arg Asn Ser Arg Gly Tyr Thr Val Leu His Leu Ala  
50 55 60

gcc atg cgc aaa gag ccg tcc atc atc ata tct ctt ctc aag aga ggt 241

Ala Met Arg Lys Glu Pro Ser Ile Ile Ile Ser Leu Leu Lys Arg Gly  
65 70 75 80

gcc aat gcg tct ggc ttc acg tgt gat gga cgc agt gcg gtt aat ata 289

Ala Asn Ala Ser Gly Phe Thr Cys Asp Gly Arg Ser Ala Val Asn Ile  
85 90 95

tgt aga aga ttg aca act cca aag gat tat cat acg aaa aca gct gcg 337

Cys Arg Arg Leu Thr Thr Pro Lys Asp Tyr His Thr Lys Thr Ala Ala  
100 105 110

aaa ggg agg gaa gct agt aaa gca cgg tta tgt ata gat ctc ttg gaa 385

Lys Gly Arg Glu Ala Ser Lys Ala Arg Leu Cys Ile Asp Leu Leu Glu  
115 120 125

aga gaa gta agg agg aac cct atg gtt gtt gat tca cca atg tgt tcc 433

Arg Glu Val Arg Arg Asn Pro Met Val Val Asp Ser Pro Met Cys Ser  
130 135 140

ctt tct atg cct gaa gat ctc caa atg aga ctg tta tac ctt gaa aat 481

Leu Ser Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu Glu Asn  
145 150 155 160

cga gtt ggc ctt gct caa ct 501

Arg Val Gly Leu Ala Gln  
165

&lt;210&gt; 52

&lt;211&gt; 166

&lt;212&gt; PRT

&lt;213&gt; Brassica napus

&lt;400&gt; 52

Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Thr Glu

1 5 10 15

Ser Asp Ile Thr Leu Asp Glu Ala Asn Gly Leu His Tyr Ser Val Val

20 25 30

Tyr Ser Asp Pro Lys Val Val Ala Glu Ile Leu Thr Leu Asp Met Gly  
 35 40 45  
 Asp Val Asn His Arg Asn Ser Arg Gly Tyr Thr Val Leu His Leu Ala  
 50 55 60  
 Ala Met Arg Lys Glu Pro Ser Ile Ile Ile Ser Leu Leu Lys Arg Gly  
 65 70 75 80  
 Ala Asn Ala Ser Gly Phe Thr Cys Asp Gly Arg Ser Ala Val Asn Ile  
 85 90 95  
 Cys Arg Arg Leu Thr Thr Pro Lys Asp Tyr His Thr Lys Thr Ala Ala  
 100 105 110  
 Lys Gly Arg Glu Ala Ser Lys Ala Arg Leu Cys Ile Asp Leu Leu Glu  
 115 120 125  
 Arg Glu Val Arg Arg Asn Pro Met Val Val Asp Ser Pro Met Cys Ser  
 130 135 140  
 Leu Ser Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu Glu Asn  
 145 150 155 160  
 Arg Val Gly Leu Ala Gln  
 165

<210> 53  
 <211> 501  
 <212> DNA  
 <213> Brassica napus

<220>  
 <221> CDS  
 <222> (2)..(499)  
 <223> Canola B

<400> 53  
 g gca ttg gat tct gat gat gtt gag ttt gtg aag ctt ctt ttg acc gag 49  
 Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Thr Glu  
 1 5 10 15  
 tca gat atc act cta gat gaa gcc aat ggt ctt cat tac tca gtg gtg 97  
 Ser Asp Ile Thr Leu Asp Glu Ala Asn Gly Leu His Tyr Ser Val Val  
 20 25 30  
 tat agt gat ccc aaa gtt gtt gcc gag att ctt act ctt gat atg ggt 145  
 Tyr Ser Asp Pro Lys Val Val Ala Glu Ile Leu Thr Leu Asp Met Gly  
 35 40 45  
 gat gtt aac cac aga aac tca cgt ggc tac acg gtt ctg cat ctc gca 193  
 Asp Val Asn His Arg Asn Ser Arg Gly Tyr Thr Val Leu His Leu Ala  
 50 55 60  
 gcc atg cgc aaa gag ccg tcc atc atc ata tct ctt ctc aag aaa ggt 241  
 Ala Met Arg Lys Glu Pro Ser Ile Ile Ile Ser Leu Leu Lys Lys Gly  
 65 70 75 80

```

gcc aat gcg tct ggc ttc acc tgt gat gga cgc agt gcg gtt aat ata 289
Ala Asn Ala Ser Gly Phe Thr Cys Asp Gly Arg Ser Ala Val Asn Ile
      85                      90                      95

tgt aga aga ttg aca act cca aag gat tat cat act aaa aca gct gcg 337
Cys Arg Arg Leu Thr Thr Pro Lys Asp Tyr His Thr Lys Thr Ala Ala
      100                      105                      110

aaa ggg agg gaa gct agt aaa gca cgg tta tgt ata gat ctc ttg gaa 385
Lys Gly Arg Glu Ala Ser Lys Ala Arg Leu Cys Ile Asp Leu Leu Glu
      115                      120                      125

aga gaa gta agg agg aac cct atg gtt gtt gag tca cca atg tgt tct 433
Arg Glu Val Arg Arg Asn Pro Met Val Val Glu Ser Pro Met Cys Ser
      130                      135                      140

ctt tct atg cct gaa gat ctc caa atg aga ctg tta tac ctt gaa aat 481
Leu Ser Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu Glu Asn
      145                      150                      155                      160

cga gtt ggc ctg gct caa ct 501
Arg Val Gly Leu Ala Gln
      165

<210> 54
<211> 166
<212> PRT
<213> Brassica napus

<400> 54
Ala Leu Asp Ser Asp Asp Val Glu Phe Val Lys Leu Leu Leu Thr Glu
  1                      5                      10                      15

Ser Asp Ile Thr Leu Asp Glu Ala Asn Gly Leu His Tyr Ser Val Val
      20                      25                      30

Tyr Ser Asp Pro Lys Val Val Ala Glu Ile Leu Thr Leu Asp Met Gly
      35                      40                      45

Asp Val Asn His Arg Asn Ser Arg Gly Tyr Thr Val Leu His Leu Ala
      50                      55                      60

Ala Met Arg Lys Glu Pro Ser Ile Ile Ile Ser Leu Leu Lys Lys Gly
      65                      70                      75                      80

Ala Asn Ala Ser Gly Phe Thr Cys Asp Gly Arg Ser Ala Val Asn Ile
      85                      90                      95

Cys Arg Arg Leu Thr Thr Pro Lys Asp Tyr His Thr Lys Thr Ala Ala
      100                      105                      110

Lys Gly Arg Glu Ala Ser Lys Ala Arg Leu Cys Ile Asp Leu Leu Glu
      115                      120                      125

Arg Glu Val Arg Arg Asn Pro Met Val Val Glu Ser Pro Met Cys Ser
      130                      135                      140

Leu Ser Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu Glu Asn
      145                      150                      155                      160

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Arg Val Gly Leu Ala Gln  
165

<210> 55  
<211> 498  
<212> DNA  
<213> Brassica napus

<220>  
<221> CDS  
<222> (2)..(496)  
<223> Canola C

<400> 55  
g gca ctg gat tct gat gat gtt gag ctt gtg aag ctt ctt ttg acc gag 49  
Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Thr Glu  
1 5 10 15  
tca gat atc act cta gat gaa gcc aat ggt ctg cat tac tca gtg gtg 97  
Ser Asp Ile Thr Leu Asp Glu Ala Asn Gly Leu His Tyr Ser Val Val  
20 25 30  
tat agt gat ccc aaa gtt gtt gca gag ata ctt gcc ctt ggt tta ggt 145  
Tyr Ser Asp Pro Lys Val Val Ala Glu Ile Leu Ala Leu Gly Leu Gly  
35 40 45  
gat gtc aat cac aga aac tca cgt ggc tac tcg gtt ctt cat ttc gct 193  
Asp Val Asn His Arg Asn Ser Arg Gly Tyr Ser Val Leu His Phe Ala  
50 55 60  
gcc atg cgt aga gag cct tcc atc atc ata tct ctt ctc aag gaa ggc 241  
Ala Met Arg Arg Glu Pro Ser Ile Ile Ile Ser Leu Leu Lys Glu Gly  
65 70 75 80  
gcc aat gcg tct agc ttc act ttt gat gga cgc agt gcg gtt aat ata 289  
Ala Asn Ala Ser Ser Phe Thr Phe Asp Gly Arg Ser Ala Val Asn Ile  
85 90 95  
tgt agg aga ctg aca act cca aag gat tat cat aca aag aca tcc aaa 337  
Cys Arg Arg Leu Thr Thr Pro Lys Asp Tyr His Thr Lys Thr Ser Lys  
100 105 110  
aag agg gaa gct agt aaa gca agg ctg tgc ata gat ctc ttg gaa aga 385  
Lys Arg Glu Ala Ser Lys Ala Arg Leu Cys Ile Asp Leu Leu Glu Arg  
115 120 125  
gag gtt agg agg aac cct atg ctt gct gat acg cca atg tgt tca ctt 433  
Glu Val Arg Arg Asn Pro Met Leu Ala Asp Thr Pro Met Cys Ser Leu  
130 135 140  
act atg cct gaa gat ctc caa atg aga ctg tta tac ctt gaa aat cga 481  
Thr Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu Glu Asn Arg  
145 150 155 160  
gtt ggt ctt gct aaa ct 498  
Val Gly Leu Ala Lys  
165

<210> 56  
 <211> 165  
 <212> PRT  
 <213> Brassica napus

<400> 56  
 Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu Leu Thr Glu  
 1 5 10 15  
 Ser Asp Ile Thr Leu Asp Glu Ala Asn Gly Leu His Tyr Ser Val Val  
 20 25 30  
 Tyr Ser Asp Pro Lys Val Val Ala Glu Ile Leu Ala Leu Gly Leu Gly  
 35 40 45  
 Asp Val Asn His Arg Asn Ser Arg Gly Tyr Ser Val Leu His Phe Ala  
 50 55 60  
 Ala Met Arg Arg Glu Pro Ser Ile Ile Ile Ser Leu Leu Lys Glu Gly  
 65 70 75 80  
 Ala Asn Ala Ser Ser Phe Thr Phe Asp Gly Arg Ser Ala Val Asn Ile  
 85 90 95  
 Cys Arg Arg Leu Thr Thr Pro Lys Asp Tyr His Thr Lys Thr Ser Lys  
 100 105 110  
 Lys Arg Glu Ala Ser Lys Ala Arg Leu Cys Ile Asp Leu Leu Glu Arg  
 115 120 125  
 Glu Val Arg Arg Asn Pro Met Leu Ala Asp Thr Pro Met Cys Ser Leu  
 130 135 140  
 Thr Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu Glu Asn Arg  
 145 150 155 160  
 Val Gly Leu Ala Lys  
 165

<210> 57  
 <211> 498  
 <212> DNA  
 <213> Brassica napus

<220>  
 <221> CDS  
 <222> (2)..(496)  
 <223> Canola D

<400> 57  
 g gca ctg gac tct gat gat gtt gag ctt gtc aag atg ctt ttg aca gaa 49  
 Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Met Leu Leu Thr Glu  
 1 5 10 15  
 gga cac acg agt cta gac gac gcc tac gct ctt cac tac gct gtt gca 97  
 Gly His Thr Ser Leu Asp Asp Ala Tyr Ala Leu His Tyr Ala Val Ala  
 20 25 30  
 cat tcc gat gtg aag acg gcc tct gat ctc ata gac ctt gag ctt gcg 145

```

His Ser Asp Val Lys Thr Ala Ser Asp Leu Ile Asp Leu Glu Leu Ala
   35                               40                               45

gat gtt gac cat aga aac ctg agg ggg tac acg gcg ctt cac gtt gct 193
Asp Val Asp His Arg Asn Leu Arg Gly Tyr Thr Ala Leu His Val Ala
   50                               55                               60

gcg atg agg aac gag ccg aag ctg atg gtt tat tta ttg act aaa ggt 241
Ala Met Arg Asn Glu Pro Lys Leu Met Val Tyr Leu Leu Thr Lys Gly
   65                               70                               75                               80

gcg aat gcg tcg gag aca acg ttt gac ggt aga acg gct ctt gtg att 289
Ala Asn Ala Ser Glu Thr Thr Phe Asp Gly Arg Thr Ala Leu Val Ile
   85                               90                               95

gca aaa aga ctc act aaa gct tct gag tat aat gct agt acg gag caa 337
Ala Lys Arg Leu Thr Lys Ala Ser Glu Tyr Asn Ala Ser Thr Glu Gln
  100                               105                               110

ggg aag cct tct ctg aaa gga ggg cta tgc ata gag gta cta gag cat 385
Gly Lys Pro Ser Leu Lys Gly Gly Leu Cys Ile Glu Val Leu Glu His
  115                               120                               125

gcg cgg aaa cta ggt agg ttg cct aga gat ggt tta cct tct ctt cca 433
Ala Arg Lys Leu Gly Arg Leu Pro Arg Asp Gly Leu Pro Ser Leu Pro
  130                               135                               140

gct act cct gat gaa ctg agg atg agg ttg ctc tac ctt gaa aat cga 481
Ala Thr Pro Asp Glu Leu Arg Met Arg Leu Leu Tyr Leu Glu Asn Arg
  145                               150                               155                               160

gtt ggc ctg gct caa ct
Val Gly Leu Ala Gln
  165

```

<210> 58  
 <211> 165  
 <212> PRT  
 <213> Brassica napus

```

<400> 58
Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Met Leu Leu Thr Glu
  1                               5                               10                               15

Gly His Thr Ser Leu Asp Asp Ala Tyr Ala Leu His Tyr Ala Val Ala
  20                               25                               30

His Ser Asp Val Lys Thr Ala Ser Asp Leu Ile Asp Leu Glu Leu Ala
  35                               40                               45

Asp Val Asp His Arg Asn Leu Arg Gly Tyr Thr Ala Leu His Val Ala
  50                               55                               60

Ala Met Arg Asn Glu Pro Lys Leu Met Val Tyr Leu Leu Thr Lys Gly
  65                               70                               75                               80

Ala Asn Ala Ser Glu Thr Thr Phe Asp Gly Arg Thr Ala Leu Val Ile
  85                               90                               95

Ala Lys Arg Leu Thr Lys Ala Ser Glu Tyr Asn Ala Ser Thr Glu Gln

```



100					105					110						
Gly	Lys	Pro	Ser	Leu	Lys	Gly	Gly	Leu	Cys	Ile	Glu	Val	Leu	Glu	His	
115					120					125						
Ala	Arg	Lys	Leu	Gly	Arg	Leu	Pro	Arg	Asp	Gly	Leu	Pro	Ser	Leu	Pro	
130					135					140						
Ala	Thr	Pro	Asp	Glu	Leu	Arg	Met	Arg	Leu	Leu	Tyr	Leu	Glu	Asn	Arg	
145					150					155					160	
Val	Gly	Leu	Ala	Gln												
165																

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<210> 59
<211> 31
<212> DNA
<213> Artificial Sequence
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<220>  
<223> Description of Artificial Sequence: PCR primer NIM  
3A

```
<400> 59
tagatgawgc mtaygctcty caytatgctg t                                     31
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```
<210> 60
<211> 32
<212> DNA
<213> Artificial Sequence
```

<220>  
<223> Description of Artificial Sequence: PCR primer NIM  
3B

```
<400> 60
ggctcyttmc kcatggcagc aayrtgaags ac 32
```

```
<210> 61
<211> 148
<212> DNA
<213> Lycopersicon esculentum
```

```
<220>  
<221> CDS  
<222> (4)..(147)  
<223> Tomato B
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<400>	61																
tag	atg	atg	cat	atg	ctc	ttc	att	atg	ctg	ttg	cat	att	gtg	acc	cca	48	
	Met	Met	His	Met	Leu	Phe	Ile	Met	Leu	Leu	His	Ile	Val	Thr	Pro		
	1				5					10					15		
agg	ttg	ttg	ctg	agg	ttc	ttg	gac	tgg	gtg	ttg	cta	atg	tca	acc	ttc	96	
Arg	Leu	Leu	Leu	Arg	Phe	Leu	Asp	Trp	Val	Leu	Leu	Met	Ser	Thr	Phe		
				20					25					30			



atc gtc gtt tcc ggt gat tcg cgt gaa gtc gcc gtt cat cgg tgt gtt	406
Ile Val Val Ser Gly Asp Ser Arg Glu Val Ala Val His Arg Cys Val	
85 90 95	
ctc tcg tct cgg agc tcg ttc ttt cgg tcc gct ttt gct tcg aaa cga	454
Leu Ser Ser Arg Ser Ser Phe Phe Arg Ser Ala Phe Ala Ser Lys Arg	
100 105 110	
gag aag gag aag gag agg gat aaa gag aga gtg gtg aag ctt gag ctt	502
Glu Lys Glu Lys Glu Arg Asp Lys Glu Arg Val Val Lys Leu Glu Leu	
115 120 125 130	
aag gat tta gct ggt gat ttt gag gtt gga ttt gat tcg gtt gtt gcg	550
Lys Asp Leu Ala Gly Asp Phe Glu Val Gly Phe Asp Ser Val Val Ala	
135 140 145	
gtt tta ggt tat ttg tat agt ggc aaa gtt agg aat ttg cct aga gga	598
Val Leu Gly Tyr Leu Tyr Ser Gly Lys Val Arg Asn Leu Pro Arg Gly	
150 155 160	
att tgt gtt tgt gtt gat gag gat tgc tct cat gaa gct tgt cgt cct	646
Ile Cys Val Cys Val Asp Glu Asp Cys Ser His Glu Ala Cys Arg Pro	
165 170 175	
gct gtt gat ttt gtt gtt gag gtt ctc tat ttg tct cac aaa ttc gag	694
Ala Val Asp Phe Val Val Glu Val Leu Tyr Leu Ser His Lys Phe Glu	
180 185 190	
att gtc gaa ttg gtt tcg ctt tat cag agg cac cta ctg gat att ctt	742
Ile Val Glu Leu Val Ser Leu Tyr Gln Arg His Leu Leu Asp Ile Leu	
195 200 205 210	
gac aag att gca cca gat gac gtt cta gta gtg tta tct gtc gct gag	790
Asp Lys Ile Ala Pro Asp Asp Val Leu Val Val Leu Ser Val Ala Glu	
215 220 225	
atg tgt gga aat gcg tgt gac gga ttg ctg gca agg tgt att gac aag	838
Met Cys Gly Asn Ala Cys Asp Gly Leu Leu Ala Arg Cys Ile Asp Lys	
230 235 240	
att gtg agg tcc gat att gac gta acc acc att gat aaa tcc ttg ccg	886
Ile Val Arg Ser Asp Ile Asp Val Thr Thr Ile Asp Lys Ser Leu Pro	
245 250 255	
cag aat gtt gtg aaa cag ata atc gac acg cga aag gaa ctt ggg ttt	934
Gln Asn Val Val Lys Gln Ile Ile Asp Thr Arg Lys Glu Leu Gly Phe	
260 265 270	
act gaa cct ggg cgt gtt gag ttt cct gat aag cat gtg aag aga ata	982
Thr Glu Pro Gly Arg Val Glu Phe Pro Asp Lys His Val Lys Arg Ile	
275 280 285 290	
cac aga gct ttg gaa tcc gat gat gta gag tta gtc aga atg ctt tta	1030
His Arg Ala Leu Glu Ser Asp Asp Val Glu Leu Val Arg Met Leu Leu	
295 300 305	
aaa gag cgc cat aca act cta gat gat gca tat gcc ctt cac tat gct	1078
Lys Glu Arg His Thr Thr Leu Asp Asp Ala Tyr Ala Leu His Tyr Ala	
310 315 320	

gtg gca cat tgt gat gcc aag acc acc acg gag ctt ctt gag ctt ggg Val Ala His Cys Asp Ala Lys Thr Thr Thr Glu Leu Leu Glu Leu Gly 325 330 335	1126
ctt gca gat gtt aat ctt aga aat cta agg ggt cac act gtg cta cat Leu Ala Asp Val Asn Leu Arg Asn Leu Arg Gly His Thr Val Leu His 340 345 350	1174
gtg gca gcc atg aga aaa gag cct aag ata att gta tcc ttg tta acc Val Ala Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu Leu Thr 355 360 365 370	1222
aag gga gcc cat ccg tct gat ata aca tca gat gat aaa aaa gca ctg Lys Gly Ala His Pro Ser Asp Ile Thr Ser Asp Asp Lys Lys Ala Leu 375 380 385	1270
cag ata gca aag aga cta aca aaa gct gtg gac ttc tat aaa act aca Gln Ile Ala Lys Arg Leu Thr Lys Ala Val Asp Phe Tyr Lys Thr Thr 390 395 400	1318
gaa caa gga aaa gat gca cca aag gat cgg ttg tgc att gaa ata ctg Glu Gln Gly Lys Asp Ala Pro Lys Asp Arg Leu Cys Ile Glu Ile Leu 405 410 415	1366
gag caa gct gaa aga aga gaa cca ttg cta gga gaa ggt tct gtt tct Glu Gln Ala Glu Arg Arg Glu Pro Leu Leu Gly Glu Gly Ser Val Ser 420 425 430	1414
ctt gca aag gca gga gat gat ctg cgt atg aag cta tta tat ctt gaa Leu Ala Lys Ala Gly Asp Asp Leu Arg Met Lys Leu Leu Tyr Leu Glu 435 440 445 450	1462
aat aga gtt gca ctt gct cgg ttg ctc ttt cca atg gaa gcg aaa gtg Asn Arg Val Ala Leu Ala Arg Leu Leu Phe Pro Met Glu Ala Lys Val 455 460 465	1510
gct atg gat att gct caa gtg gac gga act tct gaa ttc aca ttg tca Ala Met Asp Ile Ala Gln Val Asp Gly Thr Ser Glu Phe Thr Leu Ser 470 475 480	1558
aag aat ata gct gat gca cga aga aat gcg gtg gac ttg aat gag gct Lys Asn Ile Ala Asp Ala Arg Arg Asn Ala Val Asp Leu Asn Glu Ala 485 490 495	1606
ccc ttt ata ttg aaa gag gag cat ttg cag agg atg aaa gca ctg tct Pro Phe Ile Leu Lys Glu Glu His Leu Gln Arg Met Lys Ala Leu Ser 500 505 510	1654
aaa act gtt gag ctt ggc aag cgt ttc ttt cca cgc tgc tcc gat gtt Lys Thr Val Glu Leu Gly Lys Arg Phe Phe Pro Arg Cys Ser Asp Val 515 520 525 530	1702
ctt aat aag att atg gac gcc gaa gat cta tca cag ctt gca ttt tta Leu Asn Lys Ile Met Asp Ala Glu Asp Leu Ser Gln Leu Ala Phe Leu 535 540 545	1750
gga aaa gat act cca gag gaa cgg caa agg aag aga aaa cga tac ctt Gly Lys Asp Thr Pro Glu Glu Arg Gln Arg Lys Arg Lys Arg Tyr Leu 550 555 560	1798
gaa ctg caa gac gct tta act aag gct ttt aca gag gac aaa gaa gag	1846

Glu Leu Gln Asp Ala Leu Thr Lys Ala Phe Thr Glu Asp Lys Glu Glu  
 565 570 575  
 ttt gac cgt tct aca tta tca tca tca tca tca tca act cca atg ggg 1894  
 Phe Asp Arg Ser Thr Leu Ser Ser Ser Ser Ser Ser Thr Pro Met Gly  
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 agg cca tat ggt aag acc aat ttc aag agg taa ctcccttagca gctcaaagtt 1947  
 Arg Pro Tyr Gly Lys Thr Asn Phe Lys Arg  
 595 600 605  
 gcatacgcagc tcacttgtat aatattcatg tatatgtatg aaaatttctt tttgttcttc 2007  
 ccttctattg atggccacgg ttctgatctt tttggtctgt attataattt ttgaccgatt 2067  
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 ctatccgttg gagacacata ctcttgtgtg cgatgatgaa tcaatcatca gattacatta 2187  
 cagcagccat ttcttgccat attgtaattc atgtatcaag gtacaaataa atagcgtcgt 2247  
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 <213> Beta vulgaris

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 Thr Pro Asp Ala Ala Ala Leu Arg Leu Ser Glu Asn Leu Asp Ser  
 50 55 60  
 Leu Phe Gln Pro Ser Leu Ser Leu Ser Asp Ser Asp Ser Phe Ala Asp  
 65 70 75 80  
 Ala Lys Ile Val Val Ser Gly Asp Ser Arg Glu Val Ala Val His Arg  
 85 90 95  
 Cys Val Leu Ser Ser Arg Ser Ser Phe Phe Arg Ser Ala Phe Ala Ser  
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 Lys Arg Glu Lys Glu Lys Glu Arg Asp Lys Glu Arg Val Val Lys Leu  
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 Val Ala Val Leu Gly Tyr Leu Tyr Ser Gly Lys Val Arg Asn Leu Pro  
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 Arg Gly Ile Cys Val Cys Val Asp Glu Asp Cys Ser His Glu Ala Cys  
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 Arg Pro Ala Val Asp Phe Val Val Glu Val Leu Tyr Leu Ser His Lys  
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 Phe Glu Ile Val Glu Leu Val Ser Leu Tyr Gln Arg His Leu Leu Asp  
 195 200 205  
 Ile Leu Asp Lys Ile Ala Pro Asp Asp Val Leu Val Val Leu Ser Val  
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 225 230 235 240  
 Asp Lys Ile Val Arg Ser Asp Ile Asp Val Thr Thr Ile Asp Lys Ser

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Arg Ile His Arg Ala Leu Glu Ser Asp Asp Val Glu Leu Val Arg Met
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Tyr Ala Val Ala His Cys Asp Ala Lys Thr Thr Thr Glu Leu Leu Glu
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Leu Gly Leu Ala Asp Val Asn Leu Arg Asn Leu Arg Gly His Thr Val
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Leu His Val Ala Ala Met Arg Lys Glu Pro Lys Ile Ile Val Ser Leu
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Leu Thr Lys Gly Ala His Pro Ser Asp Ile Thr Ser Asp Asp Lys Lys
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Ala Leu Gln Ile Ala Lys Arg Leu Thr Lys Ala Val Asp Phe Tyr Lys
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Thr Thr Glu Gln Gly Lys Asp Ala Pro Lys Asp Arg Leu Cys Ile Glu
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Ile Leu Glu Gln Ala Glu Arg Arg Glu Pro Leu Leu Gly Glu Gly Ser
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Tyr Leu Glu Leu Gln Asp Ala Leu Thr Lys Ala Phe Thr Glu Asp Lys
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 <213> Helianthus annuus

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 <223> full-length Sunflower B cDNA sequence

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ccc cca cca tca atc ccc gag cca cgg tgc aat att gaa atc att ggc 868
Pro Pro Pro Ser Ile Pro Glu Pro Arg Ser Asn Ile Glu Ile Ile Gly
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Ser Glu Ser Asp Cys Asn Tyr Ser Asp Ala Glu Val Val Val Glu Gly
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Lys Pro Lys Tyr Asn Met Ser Asp Leu Leu Pro Tyr Gly Ser Val Gly
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tat gat gcg ttt ctc gtg ttt tta agc tat gtt tat act ggg aaa ctg 1156
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175 180 185	
cgt ctt ctc aac ttt gtg gac aag gct ctt gtt gaa gac gtg atc ccg	1348
Arg Leu Leu Asn Phe Val Asp Lys Ala Leu Val Glu Asp Val Ile Pro	
190 195 200	
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Cys Ile Asp Arg Val Val Arg Ser Lys Leu Asp Thr Ile Ser Ile Glu	
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Lys Glu Leu Pro Phe Glu Val Thr Gln Met Ile Lys Ser Ile Asp Asn	
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255 260 265	
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Arg Glu Lys Arg Ile Lys Ser Ile His Lys Ala Leu Asp Cys Asp Asp	
270 275 280	
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Val Glu Leu Val Lys Met Ile Leu Asp Glu Ser Lys Ile Thr Leu Asp	
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Ala Lys Glu Ile Leu Asn Leu Asn Arg Ala Asp Val Asn Leu Arg Asn	
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Ser Arg Asp Tyr Thr Val Leu His Val Ala Ala Met Arg Lys Glu Pro	
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Thr Phe Asp Gly Gln Ser Ala Val Ser Ile Cys Arg Arg Arg Thr Arg	
365 370 375 380	
ccc aag gat tat tat gtg aaa acc gaa cac ggg caa gaa aca aat aaa	1924
Pro Lys Asp Tyr Tyr Val Lys Thr Glu His Gly Gln Glu Thr Asn Lys	
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Met Ile Gly Asp Val Ser Val Cys Ser Ser Ala Val Ala Asp Asp Leu  
415 420 425

cat atg aat tta ctc tac tta gaa aac cga gtg gca ttt gct cga ctg 2068  
His Met Asn Leu Leu Tyr Leu Glu Asn Arg Val Ala Phe Ala Arg Leu  
430 435 440

tta ttt ccg tca gaa gcg aaa cta gca atg gaa att gcg cat gcc caa 2116  
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445 450 455 460

acg act gca cag tat ccg ggt cta ttg gca tgc aaa ggg tca aat ggt 2164  
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465 470 475

aac tta agg gag atg gat ttg aac gag aca ccg ttg gtg cag aac aaa 2212  
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aga ttg ctt tca aga atg gaa gcc ctt tcc cgg aca gtg gaa atg ggt 2260  
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caa gaa atc aaa agg acg cga ttt atg gag ctt aaa gaa gat gtc caa 2404  
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560 565 570

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575 580 585

aaa tac tca tga aacccccgtg tttctttgat gatcttttaa caagctttta 2552  
Lys Tyr Ser  
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 Cys Asn Tyr Ser Asp Ala Glu Val Val Val Glu Gly Ile Ser Val Gly  
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 Phe Lys Lys Asn Lys Gly Cys Val Glu Lys Asp Ser Lys Pro Lys Tyr  
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 Pro Glu Val Ser Thr Cys Val Asp Asp Gly Cys Leu His Asp Ala Cys  
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 210 215 220  
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 370 375 380  
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 405 410 415  
 Val Ser Val Cys Ser Ser Ala Val Ala Asp Asp Leu His Met Asn Leu

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Tyr Pro Gly Leu Leu Ala Ser Lys Gly Ser Asn Gly Asn Leu Arg Glu
465          470          475          480
Met Asp Leu Asn Glu Thr Pro Leu Val Gln Asn Lys Arg Leu Leu Ser
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Arg Met Glu Ala Leu Ser Arg Thr Val Glu Met Gly Arg Arg Tyr Phe
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Pro His Cys Ser Glu Val Leu Asp Lys Phe Met Glu Asp Asp Leu Gln
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Asp Leu Phe Ile Leu Glu Lys Gly Thr Glu Glu Glu Gln Glu Ile Lys
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Arg Thr Arg Phe Met Glu Leu Lys Glu Asp Val Gln Arg Ala Phe Thr
545          550          555          560
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Asn Leu Leu Ile Asn Gly Gln Ala Phe Ser Asp Val Thr Phe Ser Val
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gaa ggt cgt tta gtc cac gct cac cgt tgt atc ctc gcc gca cgg agg 144
Glu Gly Arg Leu Val His Ala His Arg Cys Ile Leu Ala Ala Arg Arg
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Ser	Ile	Val	Pro	Gln	Lys	His	Glu	Pro	Arg	Pro	Asn	Cys	Gly	Glu	Arg		
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Leu	Arg	Leu	Lys	Ser	Ser	Ile	Ala	Arg	Arg	Ser	Leu	Met	Pro	His	Asn		
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His	His	His	Asp	Leu	Ser	Xaa	Xaa	Gln	Xaa	Leu	Lys	Xaa	Lys	Val	Arg		
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 Leu Phe Phe Arg Lys Phe Phe Cys Gly Thr Asp Ser Pro Gln Pro Val  
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 Thr Gly Ile Asp Pro Thr Gln His Gly Ser Val Pro Ala Ser Pro Thr  
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 Tyr Glu Val Phe Leu Leu Leu Leu Gln Phe Leu Tyr Ser Gly Gln Val  
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 Ser Ile Val Pro Gln Lys His Glu Pro Arg Pro Asn Cys Gly Glu Arg  
 115 120 125  
 Gly Cys Trp His Thr His Cys Ser Ala Ala Val Asp Leu Ala Leu Asp  
 130 135 140  
 Thr Leu Ala Ala Ser Arg Tyr Phe Gly Val Glu Gln Leu Ala Leu Leu  
 145 150 155 160  
 Thr Gln Lys Gln Leu Ala Ser Met Val Glu Lys Ala Ser Ile Glu Asp  
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 Val Met Lys Val Leu Ile Ala Ser Arg Lys Gln Asp Met His Gln Leu  
 180 185 190  
 Trp Thr Thr Cys Ser His Leu Val Ala Lys Ser Gly Leu Pro Pro Glu  
 195 200 205  
 Ile Leu Ala Lys His Leu Pro Ile Asp Val Val Thr Lys Ile Glu Glu  
 210 215 220  
 Leu Arg Leu Lys Ser Ser Ile Ala Arg Arg Ser Leu Met Pro His Asn  
 225 230 235 240  
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&lt;223&gt; AtNMLC4-1 cDNA sequence

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gcc aat ctt gaa gag ctc agc tct aac ttg gag cag ctt ctc act aat	144
Ala Asn Leu Glu Glu Leu Ser Ser Asn Leu Glu Gln Leu Leu Thr Asn	
35 40 45	
cca gat tgc gat tac act gac gca gag atc atc att gaa gaa gaa gct	192
Pro Asp Cys Asp Tyr Thr Asp Ala Glu Ile Ile Ile Glu Glu Glu Ala	
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aac cct gtg agt gtt cat aga tgt gtt tta gct gct agg agc aag ttt	240
Asn Pro Val Ser Val His Arg Cys Val Leu Ala Ala Arg Ser Lys Phe	
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ttt ctt gat ctg ttt aag aaa gat aaa gat agt agt gag aag aaa cct	288
Phe Leu Asp Leu Phe Lys Lys Asp Lys Asp Ser Ser Glu Lys Lys Pro	
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Lys Tyr Gln Met Lys Asp Leu Leu Pro Tyr Gly Asn Val Gly Arg Glu	
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Ala Phe Leu His Phe Leu Ser Tyr Ile Tyr Thr Gly Arg Leu Lys Pro	
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Phe Val Phe Gln Ile Pro Asp Leu Val Ser Ser Phe Gln Arg Lys Leu	
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Leu Val Ala Phe His Cys Asp Leu Thr Gln Leu Leu Asp Gln Cys Ile	
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Glu Arg Val Ala Arg Ser Asp Leu Asp Arg Phe Cys Ile Glu Lys Glu	
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Val	Asn	Ile	Pro	Glu	Val	Glu	Asp	Lys	Ser	Ile	Glu	Arg	Thr	Gly	Lys		
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Asp	Met	Ala	Asp	Val	Asn	Phe	Arg	Asn	Ser	Arg	Gly	Tyr	Thr	Val	Leu		
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cat	att	gct	gct	atg	cgt	aga	gag	cca	aca	att	atc	ata	cca	ctt	att	1008	
His	Ile	Ala	Ala	Met	Arg	Arg	Glu	Pro	Thr	Ile	Ile	Ile	Pro	Leu	Ile		
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caa	aaa	gga	gct	aat	gct	tca	gat	ttc	acg	ttt	gat	gga	cgc	agt	gcg	1056	
Gln	Lys	Gly	Ala	Asn	Ala	Ser	Asp	Phe	Thr	Phe	Asp	Gly	Arg	Ser	Ala		
			340					345					350				
gta	aat	ata	tgt	agg	aga	ctc	act	agg	ccg	aaa	gat	tat	cat	acc	aaa	1104	
Val	Asn	Ile	Cys	Arg	Arg	Leu	Thr	Arg	Pro	Lys	Asp	Tyr	His	Thr	Lys		
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acc	tca	agg	aaa	gaa	cct	agt	aaa	tac	cgc	tta	tgc	atc	gat	atc	ttg	1152	
Thr	Ser	Arg	Lys	Glu	Pro	Ser	Lys	Tyr	Arg	Leu	Cys	Ile	Asp	Ile	Leu		
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gaa	agg	gaa	att	aga	agg	aat	cca	ttg	ggt	agt	ggg	gat	aca	ccc	act	1200	
Glu	Arg	Glu	Ile	Arg	Arg	Asn	Pro	Leu	Val	Ser	Gly	Asp	Thr	Pro	Thr		
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tgt	tcc	cat	tcg	atg	ccc	gag	gat	ctc	caa	atg	agg	ttg	tta	tac	tta	1248	
Cys	Ser	His	Ser	Met	Pro	Glu	Asp	Leu	Gln	Met	Arg	Leu	Leu	Tyr	Leu		
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Glu	Lys	Arg	Val	Gly	Leu	Ala	Gln	Leu	Phe	Phe	Pro	Ala	Glu	Ala	Asn		
			420				425						430				
gtg	gct	atg	gac	ggt	gct	aat	ggt	gaa	ggg	aca	agc	gag	tgc	aca	ggt	1344	
Val	Ala	Met	Asp	Val	Ala	Asn	Val	Glu	Gly	Thr	Ser	Glu	Cys	Thr	Gly		
			435			440						445					
ctt	cta	act	cca	cct	cca	tca	aat	gat	aca	act	gaa	aac	ttg	ggt	aaa	1392	
Leu	Leu	Thr	Pro	Pro	Pro	Ser	Asn	Asp	Thr	Thr	Glu	Asn	Leu	Gly	Lys		
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gtc	gat	tta	aat	gaa	acg	cct	tat	gtg	caa	acg	aaa	aga	atg	ctt	aca	1440	
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Arg Met Lys Ala Leu Met Lys Thr Val Glu Thr Gly Arg Arg Tyr Phe	485	490	495	
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Pro Ser Cys Tyr Glu Val Leu Asp Lys Tyr Met Asp Gln Tyr Met Asp	500	505	510	
gaa gaa atc cct gat atg tcg tat ccc gag aaa ggc act gtg aaa gag				1584
Glu Glu Ile Pro Asp Met Ser Tyr Pro Glu Lys Gly Thr Val Lys Glu	515	520	525	
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Arg Arg Gln Lys Arg Met Arg Tyr Asn Glu Leu Lys Asn Asp Val Lys	530	535	540	
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Lys Ala Tyr Ser Lys Asp Lys Val Ala Arg Ser Cys Leu Ser Ser Ser	545	550	555	560
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&lt;213&gt; Arabidopsis thaliana

&lt;400&gt; 70

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Pro Asp Cys Asp Tyr Thr Asp Ala Glu Ile Ile Ile Glu Glu Glu Ala	
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145 150 155 160	
Phe Val Phe Gln Ile Pro Asp Leu Val Ser Ser Phe Gln Arg Lys Leu	
165 170 175	
Arg Asn Tyr Val Glu Lys Ser Leu Val Glu Asn Val Leu Pro Ile Leu	
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Leu Val Ala Phe His Cys Asp Leu Thr Gln Leu Leu Asp Gln Cys Ile	
195 200 205	
Glu Arg Val Ala Arg Ser Asp Leu Asp Arg Phe Cys Ile Glu Lys Glu	
210 215 220	



Leu Pro Leu Glu Val Leu Glu Lys Ile Lys Gln Leu Arg Val Lys Ser  
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 290 295 300  
 Asp Met Ala Asp Val Asn Phe Arg Asn Ser Arg Gly Tyr Thr Val Leu  
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 Val Asn Ile Cys Arg Arg Leu Thr Arg Pro Lys Asp Tyr His Thr Lys  
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 Glu Arg Glu Ile Arg Arg Asn Pro Leu Val Ser Gly Asp Thr Pro Thr  
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 Cys Ser His Ser Met Pro Glu Asp Leu Gln Met Arg Leu Leu Tyr Leu  
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 Glu Lys Arg Val Gly Leu Ala Gln Leu Phe Phe Pro Ala Glu Ala Asn  
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 Arg Met Lys Ala Leu Met Lys Thr Val Glu Thr Gly Arg Arg Tyr Phe  
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 Pro Ser Cys Tyr Glu Val Leu Asp Lys Tyr Met Asp Gln Tyr Met Asp  
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 Arg Arg Gln Lys Arg Met Arg Tyr Asn Glu Leu Lys Asn Asp Val Lys  
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Ala Glu Ser Ser Leu Asp Tyr Pro Thr Glu Phe Leu Thr Pro Pro Glu	
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Val Ser Ala Leu Lys Leu Leu Ser Asn Cys Leu Glu Ser Val Phe Asp	
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Ser Pro Glu Thr Phe Tyr Ser Asp Ala Lys Leu Val Leu Ala Gly Gly	
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Val Ala Cys Arg Ser Lys Val Asp Phe Met Val Glu Val Leu Tyr Leu	
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175 180 185	
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Phe Lys Leu Asp Thr Leu Cys Gly Thr Thr Tyr Lys Lys Leu Leu Asp	
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225 230 235	
gag aag tct tta cct caa cac att ttc aag caa atc ata gac atc cgc	771
Glu Lys Ser Leu Pro Gln His Ile Phe Lys Gln Ile Ile Asp Ile Arg	
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Glu Ala Leu Cys Leu Glu Pro Pro Lys Leu Glu Arg His Val Lys Asn	
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270 275 280 285	
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Leu Leu Glu Gly His Thr Asn Leu Asp Glu Ala Tyr Ala Leu His Phe	
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gag ctt gcg gat gtt aac ctt aga aat ccg agg gga tac act gtg ctt	1011
Glu Leu Ala Asp Val Asn Leu Arg Asn Pro Arg Gly Tyr Thr Val Leu	
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tta gtg att gta aaa cga ctc act aaa gcg gat gac tac aaa act agt	1155
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400 405 410	
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Leu Ser Leu Pro Val Thr Pro Glu Glu Leu Arg Met Arg Leu Leu Tyr	
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Tyr Glu Asn Arg Val Ala Leu Ala Arg Leu Leu Phe Pro Val Glu Thr	
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450 455 460	
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Leu Asp Leu Asn Met Ala Pro Phe Gln Ile His Glu Lys His Leu Ser	
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 aaa cga tgt tgc ctt gat cac ttt atg gat act gag gac ttg aat cat 1587  
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 Thr Phe Tyr Ser Asp Ala Lys Leu Val Leu Ala Gly Gly Arg Glu Val  
 65 70 75 80  
 Ser Phe His Arg Cys Ile Leu Ser Ala Arg Ile Pro Val Phe Lys Ser  
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 Ala Leu Ala Thr Val Lys Glu Gln Lys Ser Ser Thr Thr Val Lys Leu  
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 Val Ala Val Leu Ala Tyr Val Tyr Ser Gly Arg Val Arg Ser Pro Pro  
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 Lys Gly Ala Ser Ala Cys Val Asp Asp Asp Cys Cys His Val Ala Cys  
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 Arg Ser Lys Val Asp Phe Met Val Glu Val Leu Tyr Leu Ser Phe Val  
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 Phe Gln Ile Gln Glu Leu Val Thr Leu Tyr Glu Arg Gln Phe Leu Glu  
 180 185 190  
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 195 200 205  
 Asp Thr Leu Cys Gly Thr Thr Tyr Lys Lys Leu Leu Asp Arg Cys Ile

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Cys Leu Glu Pro Pro Lys Leu Glu Arg His Val Lys Asn Ile Tyr Lys		
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Gly His Thr Asn Leu Asp Glu Ala Tyr Ala Leu His Phe Ala Ile Ala		
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His Cys Ala Val Lys Thr Ala Tyr Asp Leu Leu Glu Leu Glu Leu Ala		
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Gly Thr Pro Ser Leu Lys Gly Gly Leu Cys Ile Glu Val Leu Glu His		
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tttttttagcc gaagtgaatg ttattccaat tgggtaagct gtgatcaagc agttgaagtt 180
ttttgttgca aaatttgcca gttatcttga ctttttgtga agttggtaaa tttttcattt 240
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gttttgttga ttatagatgg ggtggaattg ttaatttctt ctaaagtttt aaagggttga 360
tttggtttta cctgaaatag ggagaatatg acttgtagtt ttggaatttg cttcttttct 420
tgggtctgcat agttgaatgt tattagaaaa cttatggaaa gtttttgtca aacttttgtc 480
ctttgagaag aatttcttgt attggtgatt ggttatggtc ttggagaggt tctttttttt 540
tttgcataga gcctgtgcgg agaataattat acatgggttaa aaacattaga ttttctggac 600
tttgactatc ttatagtag ataaattttg tatatgtttt tagaccatta gaattgggaa 660

atg gct tgt tct gct gaa cca tca tca tct ata agc ttt act tca tct 708
Met Ala Cys Ser Ala Glu Pro Ser Ser Ser Ile Ser Phe Thr Ser Ser
  1          5          10          15

tcc att aca tcg aat ggg tcg att ggc gtt ggc caa aac act cat gct 756
Ser Ile Thr Ser Asn Gly Ser Ile Gly Val Gly Gln Asn Thr His Ala
          20          25          30

tat ggc ggc tct gag aca ggg agt agt tat gaa atc atc agc ttg agt 804
Tyr Gly Gly Ser Glu Thr Gly Ser Ser Tyr Glu Ile Ile Ser Leu Ser
          35          40          45

aaa ctc agt aac aat tta gag caa ctc ttg tca gat tcc agc tct gat 852
Lys Leu Ser Asn Asn Leu Glu Gln Leu Leu Ser Asp Ser Ser Ser Asp
          50          55          60

ttt act gat gct gag att gtt gtt gag ggt gtt tca ctt ggt gtt cac 900
Phe Thr Asp Ala Glu Ile Val Val Glu Gly Val Ser Leu Gly Val His
          65          70          75          80

cgt tgt ata tta gct gcc agg agt aaa ttt ttt cag gat ctt ttt agg 948
Arg Cys Ile Leu Ala Ala Arg Ser Lys Phe Phe Gln Asp Leu Phe Arg
          85          90          95

aaa gag aag gga agt tgt gga aag gaa ggt aaa cca aga tat tct atg 996
Lys Glu Lys Gly Ser Cys Gly Lys Glu Gly Lys Pro Arg Tyr Ser Met
          100          105          110

acc gat att ttg cct tat ggt aag gtt gga tat gag gct ttc gtt acc 1044
Thr Asp Ile Leu Pro Tyr Gly Lys Val Gly Tyr Glu Ala Phe Val Thr
          115          120          125

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Phe Leu Ser Tyr Leu Tyr Ser Gly Lys Leu Lys His Phe Pro Pro Glu	
130 135 140	
gta tca aca tgt atg gac act ata tgt gct cat gac tct tgc aga cca	1140
Val Ser Thr Cys Met Asp Thr Ile Cys Ala His Asp Ser Cys Arg Pro	
145 150 155 160	
gca att aat ttt agt gtg gag ttg atg tat gcc tct tcc atg ttt cag	1188
Ala Ile Asn Phe Ser Val Glu Leu Met Tyr Ala Ser Ser Met Phe Gln	
165 170 175	
gtt cca gag cta gta tca ctt ttc ctg aga cgc ctt atc aat ttt gtt	1236
Val Pro Glu Leu Val Ser Leu Phe Leu Arg Arg Leu Ile Asn Phe Val	
180 185 190	
ggg aag gct ctt gtg gaa gat gtt atc cca ata ctt aga gtt gct ttt	1284
Gly Lys Ala Leu Val Glu Asp Val Ile Pro Ile Leu Arg Val Ala Phe	
195 200 205	
cat tgc caa ttg agc gag ctt ctc act cat tcc gtt gat aga gta gca	1332
His Cys Gln Leu Ser Glu Leu Leu Thr His Ser Val Asp Arg Val Ala	
210 215 220	
cga tca gat ctt gaa atc aca tgc att gag aaa gag gtt ccc ttt gaa	1380
Arg Ser Asp Leu Glu Ile Thr Cys Ile Glu Lys Glu Val Pro Phe Glu	
225 230 235 240	
gtt gca gag aat att aaa tta ttg tgg ccg aaa tgt cag gtt gat gaa	1428
Val Ala Glu Asn Ile Lys Leu Leu Trp Pro Lys Cys Gln Val Asp Glu	
245 250 255	
agt aag gtt cta cct gtg gat ccc ttg cat gaa aag aga aaa aat agg	1476
Ser Lys Val Leu Pro Val Asp Pro Leu His Glu Lys Arg Lys Asn Arg	
260 265 270	
ata tac aag gca ttg gat tgc gat gat gtt gaa ctt gtc aag ctt cta	1524
Ile Tyr Lys Ala Leu Asp Ser Asp Asp Val Glu Leu Val Lys Leu Leu	
275 280 285	
ctg agt gag tct aac ata agc tta gat gaa gcc tac gct ctt cat tat	1572
Leu Ser Glu Ser Asn Ile Ser Leu Asp Glu Ala Tyr Ala Leu His Tyr	
290 295 300	
gct gtg gca tat tgt gat ccc aag gtt gtg act gag gtt ctt gga ctg	1620
Ala Val Ala Tyr Cys Asp Pro Lys Val Val Thr Glu Val Leu Gly Leu	
305 310 315 320	
ggt gtt gcg gat gtc aac cta cgt aat act cgt ggt tac act gtg ctt	1668
Gly Val Ala Asp Val Asn Leu Arg Asn Thr Arg Gly Tyr Thr Val Leu	
325 330 335	
cac att gct tcc atg cgt aag gag cca gca gta att gta tgc ctt ttg	1716
His Ile Ala Ser Met Arg Lys Glu Pro Ala Val Ile Val Ser Leu Leu	
340 345 350	
act aag gga gct cgt gca tca gag act aca ttg gat ggg cag agt gct	1764
Thr Lys Gly Ala Arg Ala Ser Glu Thr Thr Leu Asp Gly Gln Ser Ala	
355 360 365	
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Val

acaagggccag gaagcaaaca aagatcgggt atgtattgat gttttggaga gagagatgcg 1877  
 tcgcaaccca atggctggag atgcattgtt ttcttcccca atgttggccg atgatctgca 1937  
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&lt;210&gt; 74

&lt;211&gt; 369

&lt;212&gt; PRT

<213> *Nicotiana tabacum*

&lt;400&gt; 74

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Tyr	Gly	Gly	Ser	Glu	Thr	Gly	Ser	Ser	Tyr	Glu	Ile	Ile	Ser	Leu	Ser	35	40	45	
Lys	Leu	Ser	Asn	Asn	Leu	Glu	Gln	Leu	Leu	Ser	Asp	Ser	Ser	Ser	Asp	50	55	60	
Phe	Thr	Asp	Ala	Glu	Ile	Val	Val	Glu	Gly	Val	Ser	Leu	Gly	Val	His	65	70	75	80
Arg	Cys	Ile	Leu	Ala	Ala	Arg	Ser	Lys	Phe	Phe	Gln	Asp	Leu	Phe	Arg	85	90	95	
Lys	Glu	Lys	Gly	Ser	Cys	Gly	Lys	Glu	Gly	Lys	Pro	Arg	Tyr	Ser	Met	100	105	110	
Thr	Asp	Ile	Leu	Pro	Tyr	Gly	Lys	Val	Gly	Tyr	Glu	Ala	Phe	Val	Thr				



115					120					125					
Phe	Leu	Ser	Tyr	Leu	Tyr	Ser	Gly	Lys	Leu	Lys	His	Phe	Pro	Pro	Glu
130					135					140					
Val	Ser	Thr	Cys	Met	Asp	Thr	Ile	Cys	Ala	His	Asp	Ser	Cys	Arg	Pro
145					150					155					160
Ala	Ile	Asn	Phe	Ser	Val	Glu	Leu	Met	Tyr	Ala	Ser	Ser	Met	Phe	Gln
				165					170					175	
Val	Pro	Glu	Leu	Val	Ser	Leu	Phe	Leu	Arg	Arg	Leu	Ile	Asn	Phe	Val
			180					185					190		
Gly	Lys	Ala	Leu	Val	Glu	Asp	Val	Ile	Pro	Ile	Leu	Arg	Val	Ala	Phe
		195					200					205			
His	Cys	Gln	Leu	Ser	Glu	Leu	Leu	Thr	His	Ser	Val	Asp	Arg	Val	Ala
	210					215					220				
Arg	Ser	Asp	Leu	Glu	Ile	Thr	Cys	Ile	Glu	Lys	Glu	Val	Pro	Phe	Glu
225					230					235					240
Val	Ala	Glu	Asn	Ile	Lys	Leu	Leu	Trp	Pro	Lys	Cys	Gln	Val	Asp	Glu
			245						250					255	
Ser	Lys	Val	Leu	Pro	Val	Asp	Pro	Leu	His	Glu	Lys	Arg	Lys	Asn	Arg
			260					265					270		
Ile	Tyr	Lys	Ala	Leu	Asp	Ser	Asp	Asp	Val	Glu	Leu	Val	Lys	Leu	Leu
		275					280					285			
Leu	Ser	Glu	Ser	Asn	Ile	Ser	Leu	Asp	Glu	Ala	Tyr	Ala	Leu	His	Tyr
	290					295					300				
Ala	Val	Ala	Tyr	Cys	Asp	Pro	Lys	Val	Val	Thr	Glu	Val	Leu	Gly	Leu
305					310					315					320
Gly	Val	Ala	Asp	Val	Asn	Leu	Arg	Asn	Thr	Arg	Gly	Tyr	Thr	Val	Leu
			325						330					335	
His	Ile	Ala	Ser	Met	Arg	Lys	Glu	Pro	Ala	Val	Ile	Val	Ser	Leu	Leu
			340					345					350		
Thr	Lys	Gly	Ala	Arg	Ala	Ser	Glu	Thr	Thr	Leu	Asp	Gly	Gln	Ser	Ala
		355					360					365			
Val															